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Placental Transfer and Fetal Tissue Uptake of Mg^{28} in the Rabbit.* (26020)

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The factors regulating the metabolism of magnesium are obscure and very little is known about the placental transfer of this ion or its uptake in the fetal tissues. The purpose of the present study in rabbits was to investigate, by use of a radioactive isotope of magnesium (Mg^{28}), the maternal-to-fetal transfer of magnesium and uptake of the isotope by various maternal and fetal tissues.

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Material and methods. Pregnant domestic albino rabbits, between 28 and 30 days of gestation, were anesthetized with sodium pentobarbital given intravenously in a dosage of 40 mg/kg of body weight. After a tracheotomy had been performed, the carotid artery was cannulated and an indwelling polyethylene catheter, connected to a reservoir of heparin, was inserted.

Mg^{28} was then injected into the marginal vein of the ear as a solution of magnesium sulfate, prepared according to a method previ-

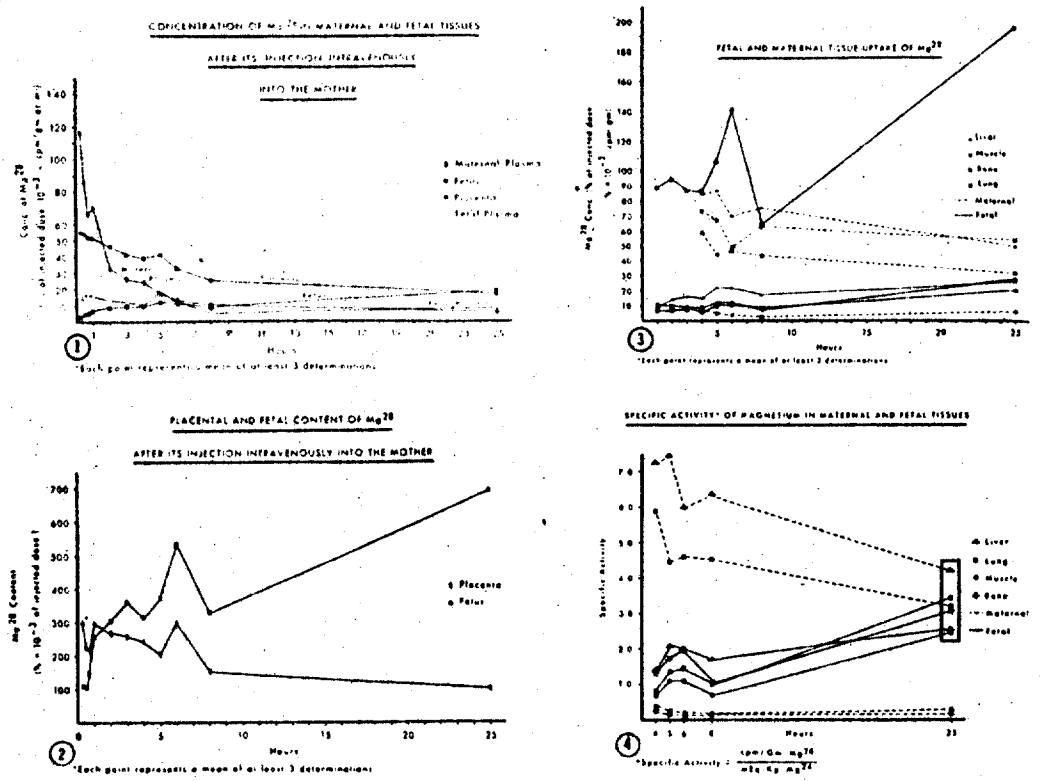

FIG. 1-4.

ously described(1): 20 μc of Mg^{28} was contained in 2 meq of stable magnesium.

Abdominal cavity was exposed by a midline incision. At intervals varying from 7 minutes to 26 hours after injection of Mg^{28}, a fetus and its placenta were removed from the uterus. A wide V-shaped incision was made over the thorax of the fetus and blood was obtained by cardiac puncture with a 20-gauge hypodermic needle attached to a heparinized syringe. Simultaneously, with withdrawal of fetal blood, a maternal blood specimen was obtained from the carotid artery. At termination of each experiment, the mother was killed by air embolism and samples of tissues were obtained for assays of radioactivity and magnesium content.

Serum and tissue magnesium determinations were performed by the molybdivanadate method for phosphate(2).

Radioactivity assay. Samples of plasma and tissues were assayed for gamma ray activity in a well-type scintillation counter. Total Mg^{28} content of each fetus and placenta was determined; samples of liver, kidney, skeletal muscle, tibia and femur, and skull were then removed from each fetus and their concentrations of radioactivity determined separately. All results are expressed as percentages of total administered dose of radioactivity.

Results. A total of 86 fetuses and their respective placentas were obtained from 11 pregnant rabbits. Clearance of Mg^{28} from the maternal circulation, its concentration in placenta and fetus, and appearance of Mg^{28} in fetal circulation are summarized in Fig. 1.

Changes in concentration of Mg^{28}. Concentration of Mg^{28} in maternal plasma decreased rapidly during the first 2 hours; thereafter, the decline was slower, but progressive. Concentration of Mg^{28} was initially lower in the placenta than in the maternal plasma, but within 2 hours, placental concentration became higher and remained higher thereafter.

The progressive decrease of Mg^{28} in the placenta paralleled that in the maternal plasma. Concentration of Mg^{28} in the fetus was initially lower than that in maternal plasma or placenta; it increased gradually, however, and at 24 hours exceeded both maternal and placental concentration of the isotope.

Changes in Mg^{28} content. Average fetal content of Mg^{28} increased progressively (Fig. 2). Between the first and second hour it surpassed the placental content and remained higher thereafter. As fetal content of Mg^{28} increased, that in the placenta decreased progressively.

Tissue distribution of Mg^{28} (Fig. 3). Of all the *maternal* tissues studied, uptake of Mg^{28} was lowest in the muscle. Successively higher concentrations of Mg^{28} were found in lung, long bone, liver and kidney. Concentration of Mg^{28} in all maternal tissues tended to decrease progressively with time as concentration in the fetal tissues increased.

Relative concentrations of Mg^{28} in *fetal* tissues remained consistently in the following order: (1) lung (lowest), (2) muscle, liver and kidney (intermediate) and (3) bone (highest). Comparison of tissue concentrations of Mg^{28} in mother and fetus at various time intervals revealed that concentrations of Mg^{28} in the kidney, lung and liver were higher in the mother than in the fetus. Mg^{28} concentrations in fetal muscle and bone, however, were much higher than maternal concentrations.

The maternal and fetal *tissue contents* of magnesium are summarized in Table I. Magnesium content of all the tissues studied was lower in the fetus than in the mother, the most notable difference being bone.

Specific activities of maternal and fetal liver, lung, muscle and bone are summarized in Fig. 4. By 26 hours, all values, except those for maternal bone and muscle, appeared to reach a constant level.

TABLE I. Fetal and Maternal Tissue Content of Magnesium.

Tissue	Muscle	Kidney	Liver	Lung	Bone
Mother	19.8*	18.5	11.7	10.0	298.1
Fetus	10.8	11.2	10.4	7.9	62.9

* meq/kg (wet wt) of tissue.

Comment. When Mg^{28} was injected intravenously into pregnant rabbits, its clearance from the blood stream was similar to that previously observed in healthy young adult rabbits(3). Its distribution in tissues of the mothers was also similar to that in nonpregnant animals, except for slower uptake in bone and muscle of pregnant rabbits. Previous studies have shown that, in pregnancy, magnesium is mobilized from maternal tissues (4). In addition, bone uptake of Mg^{28} tends to decrease with age. Both of these factors probably contributed to the low uptake of Mg^{28} by maternal bone and muscle.

The observation that placental concentration of Mg^{28} at 2 hours was higher than concentration in maternal plasma suggests that the placenta actively concentrates magnesium. Because of the low specific activity of the Mg^{28} available for this study, the mother was subjected to a slight magnesium load. This maternal loading may have affected the placental concentration of Mg^{28}.

Comparison of tissue magnesium content in mother and fetus shows a higher concentration of untagged magnesium in all maternal tissues studied. In spite of the lower magnesium concentration of fetal tissues, Mg^{28} uptake was rapid and increased progressively. The fact that the specific activities of all fetal tissues and of maternal liver, kidney and lung approached a constant value at 24 hours suggests that Mg^{28} was at equilibrium with the untagged element in these tissues by this time. It is of interest that the specific activity of fetal muscle and bone was considerably higher than that of the respective maternal tissues. Previous studies support the interpretation that rabbit fetuses at this stage of development are rapidly increasing in bone and muscle mass and, hence, turnover of magnesium in these tissues is more rapid than that in the respective maternal tissues.

Our results show that Mg^{28}, injected intravenously into the mother near term, rapidly crosses the placenta and, because of the more rapid growth of the fetus, is concentrated in the fetal tissues. These results further suggest that rate of uptake of magnesium by various tissues is related to anabolic activities of the cells involved. In comparison with the

cellular exchange of potassium, for instance, transport of magnesium across the cell membrane is a relatively slow process.

Summary. Mg^{28} was injected intravenously into 11 pregnant rabbits between 28 and 30 days of gestation. Fetuses and placentas were removed at intervals ranging from 7 minutes to 26 hours. Maternal tissue uptake of Mg^{28} resembled that previously found in nonpregnant young adult rabbits, except that uptake in bone and muscle was slower. Concentration of Mg^{28} in the placenta rapidly rose above the maternal plasma level. Specific activity of magnesium in all fetal tissues studied reached a fairly constant value by 26 hours. Magnesium turnover in tissues of the fetus *in utero*, especially in bone and muscle, is considerably more rapid than that in the respective tissues of the mother.

Mg^{28} was supplied by Brookhaven Laboratory on allocation from U. S. Atomic Energy Comm.

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TECHNICAL SECTION

FLUOROMETRIC ESTIMATION OF MAGNESIUM IN SERUM AND URINE

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Although magnesium is a major intracellular cation, implicated in a large variety of biochemical reactions, our knowledge of its metabolism is quite deficient in contrast with that of sodium, potassium, and calcium. This deficiency results chiefly from the fact that magnesium is difficult to measure and analyze in biologic fluids.

The bases for the most frequently used procedures for the assay of magnesium are: precipitation as magnesium ammonium phosphate or as magnesium quinolate,[15] colorimetric estimation with titan yellow,[3, 15] complexometric titration with ethylenediaminetetraacetic acid,[6, 7] emission flame,[2, 25, 39] or atomic absorption flame spectrophotometry,[11] and fluorometric measurements.[5, 28, 29]

Although it is considered to be an "extremely sensitive" method of chemical analysis,[1] the fluorometric estimation of magnesium has not been widely used in the clinical laboratory. There are only a few reports of normal values of serum magnesium estimated by fluorometric means,[5, 25, 29] and we have been unable to find any reference to fluorometrically estimated urine magnesium values.

It is the authors' purpose in this paper to (1) describe the ranges and means of magnesium in normal serum and urine, as obtained by means of a fluorometric measurement, and (2) include data pertaining to the validity, specificity, and reproducibility of fluorometric estimations of magnesium in serum and urine.

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METHODS AND MATERIALS

In order to demonstrate and evaluate reproducibility of results of the fluorometric estimation of magnesium in serum and urine, the study was performed not only with *duplicate* measurements, but also in 2 separate clinical chemistry laboratories, the Section of Clinical Chemistry at The University of Michigan Medical Center, and the chemistry section of the Clinical Laboratories, St. Joseph Mercy Hospital, Ann Arbor, Michigan. Comparison with a titan yellow method was performed in the latter laboratory.

Normal serum studies were performed on serums from 113 blood donors to the blood banks of the aforementioned institutions.

Normal urine studies were performed on 24-hr. collections from 46 freshman medical students at The University of Michigan Medical School. No dietary control or restrictions were imposed.

The possible interfering effects of various cations upon the fluorescence in serum as well as urine were studied by means of the addition or substitution of the respective cation. Finally, recovery of added magnesium to urine was studied.

Estimation of the serum and urine magnesium was performed according to the method of Schachter,[25] using a Turner fluorimeter.* Reagents were prepared as indicated by Schachter.[25] The *magnesium standard* (2 mg./100 ml.) was prepared by dissolving 176.4 mg. of reagent grade crystalline magnesium acetate in water diluted to 1 l. This standard is stable for at least 6 months if stored at room temperature in a dry polyethylene bottle. The *oxine stock solution*, prepared by dissolving 5 Gm. of

*Model No. 110, G. K. Turner Associates, 2524 Pulgas Avenue, Palo Alto, California.

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reagent grade 8-hydroxyquinoline (free base) in 100 ml. of ethanol is stable for 4 weeks, stored at 4 C. in a brown bottle. The *acetate buffers*, 2 M, pH *3.5* and *6.5*, may be stored at room temperature for at least 2 months. The pH 3.5 buffer was prepared by adding 12.1 Gm. (11.5 ml.) reagent grade glacial acetic acid to 70 ml. of water and diluting to 100 ml. with water. Adjustment of pH is made with 2 N sodium hydroxide. The pH 6.5 buffer was prepared by dissolving 27.2 Gm. of crystalline reagent grade sodium acetate in 70 ml. of water. The pH of *this* buffer is critical as minor variations have a significant effect on the fluorescence of the magnesium oxine complex. Adjustment of pH is made by the addition of glacial or dilute acetic acid. Usually less than 1 ml. of glacial acetic acid is necessary for the adjustment. The *versenate reagent* needed for the estimation of magnesium in the urine was prepared by dissolving 3.72 Gm. of reagent grade disodium ethylenediaminetetraacetate dehydrate (EDTA) in 1 l. of water. Deionized water must be used throughout the procedure.

RESULTS

Serum. Figure 1 is a frequency histogram of the distribution of values for serum magnesium in 113 normal blood donors. The mean value is 1.88 mEq. per l., and the distribution is gaussian. The standard deviation from the mean was 0.40 mEq. per l. These values compare favorably with more elaborate, expensive, and operose technics (Table 1), and the ±S.D. range is almost identical with the 1.5 to 2.4 mEq. per l. normal range recently reported in the *British Medical Journal*.[4] Duplication and reproducibility of results were excellent, not exceeding 0.2 mEq. per l. The results in Table 2 illustrate the reproducibility of the method, and also provide a comparison of the fluorometric estimated value with those obtained on the same sample by means of a titan yellow method[3] in 15 of the serums.

The effects of various cations on estimation of magnesium by fluorometric means are listed in Table 3. It may be noted that the ions are, in most instances, far in excess of the range normally found in serum and

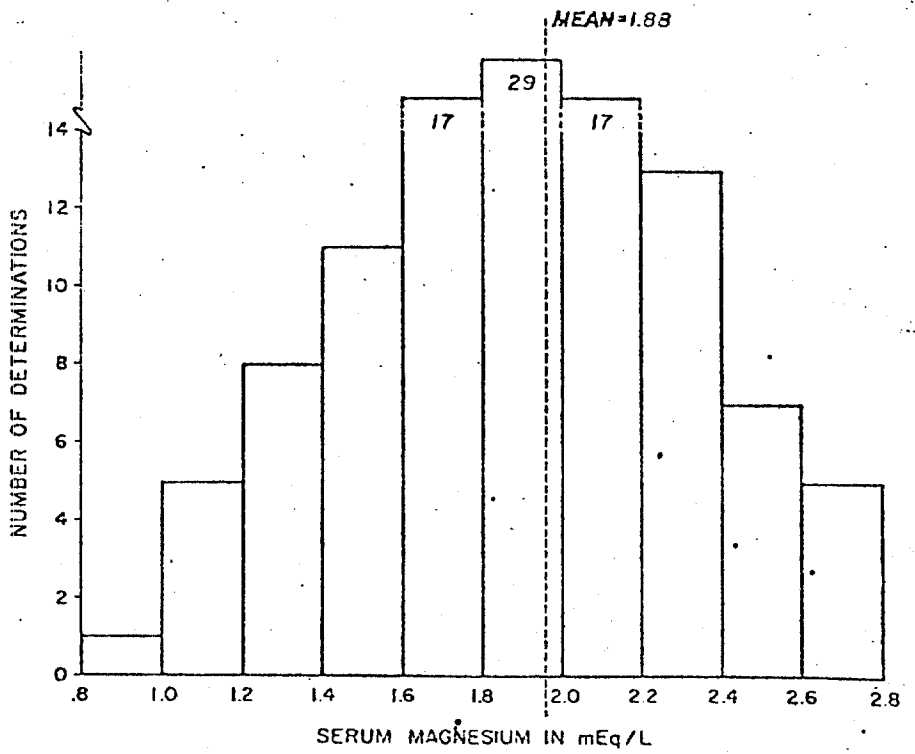

FIG. 1. Frequency histogram of the distribution of values for serum magnesium in 113 normal blood donors.

TABLE 1

MEAN LEVELS OF MAGNESIUM OF NORMAL SERUMS REPORTED BY VARIOUS AUTHORS

Authors	Year	Method*	Mean
			mEq./l.
Watchorn and McCance[41]	1932	P. P.	2.07
Hald[11]	1933	P. P.	1.70
Cope and Wolff[8]	1942	P. P.	1.66
Simonsen and associates[31]	1947	P. P.	1.68
Walker and Walker[42]	1936	P. P.	1.82
Stutzman and Amatuzio[34]	1953	P. P.	1.82
Greenberg and associates[13]	1933	H. P.	2.28
Hoffman[21]	1937	H. P.	1.82
Hirschfelder and Haury[20]	1938	T. Y.	1.76
Silverman and Gardner[30]	1954	T. Y.	1.77
Kunkel and associates[23]	1947	T. Y.	1.77
Orange and Rhein[27]	1951	T. Y.	1.87
Moscovic[26]	1962	T. Y.	1.76
Haury and Cantarow[17]	1942	T. Y.; P. P.	2.05
Carr and Frank[6]	1956	EDTA	1.72
Wallach and associates[43]	1962	EDTA	2.00
Hunter[22]	1958	E. B.	1.69
Smith[32]	1955	E. B.	1.73
Davis[10]	1955	F. P.	1.66
Van Fossan and associates[39]	1959	F. P.	1.67
Alcock and associates[2]	1960	F. P.	1.66
Hanna[15]	1961	F. P.	1.66
Wacker and Vallee[41]	1958	F. P.	2.00
Vallee and associates[39]	1960	F. P.	2.00
Montgomery[25]	1961	F. P.	1.70
Stewart and associates[33]	1963	A. Ab.	1.74
Schachter[28]	1959	F	2.05
Schachter[29]	1961	F	1.80
This report	1964	F	1.88

* The abbreviations used are: P. P., phosphate precipitation methods; H. P., hydroxyquinoline precipitation method; T. Y., titan yellow; EDTA, titration with EDTA; E. B., eriochrome black T, colorimetric; F. P., flame photometric; A. Ab., atomic absorption spectrophotometric; F, fluorometric.

urine. Only zinc produces significant fluorescence to obviate estimation of magnesium.

Urine. The distribution of magnesium values in 24-hr. collections of urine from 46 apparently normal medical students was *not* gaussian and was over a relatively broad range. The mean value at 1 of our institutions was 5.00 mEq./24 hr.; at the other laboratory the mean value was 6.05 mEq./24 hr. The range was 1.35 to 14.00 mEq./24 hr. Duplicity of results was not as good as that obtained in serum. The mean difference between duplicate readings was 0.47 mEq. with the greatest divergence being 2.00 mEq. between 2 readings on the same sample. Table 3 demonstrates that, as in serum, the presence of cations other than

TABLE 2

COMPARISON OF VALUES OBTAINED BY MEANS OF DUPLICATE FLUOROMETRIC ESTIMATIONS WITH THOSE OBTAINED BY MEANS OF TITAN YELLOW ESTIMATION

Fluorometric		Titan Yellow*
1	2	
mEq./l.		*mEq./l.*
1.90	1.90	2.16
1.85	1.85	2.00
2.30	2.25	2.50
2.20	2.10	2.20
1.50	1.50	1.70
1.85	1.90	2.00
1.95	2.00	2.22
1.85	1.75	2.00
1.50	1.40	1.70
1.85	1.80	1.80
1.75	1.65	2.00
1.30	1.30	3.20
2.00	1.90	2.30
2.05	2.00	1.80
2.10	2.30	1.82

TABLE 3

EFFECT OF VARIOUS CATIONS ON THE FLUOROMETRIC ESTIMATION OF MAGNESIUM IN SERUM AND URINE

Cation	Amount	Increase in Estimated Value of Magnesium
	mg./100 ml.	
Serum		
Sodium	100	0.53
Potassium	100	0.47
Calcium	10	0.06
Calcium	100	0.45
Zinc	50	>4.0
Urine		
Sodium	100	0.00
Potassium	100	0.00
Calcium	25	0.00
Phosphorus	70	0.01
Zinc	2.5	1.66
Zinc	10	3.45

TABLE 4

RECOVERIES OF MAGNESIUM ADDED TO NORMAL URINE

Magnesium Added*	Recovery
mg./100 ml.	%
5.0	105.0
10.0	94.6
20.0	88.0
35.0	93.0

* Indicated amounts were added as $MgCl_2$.

zinc does not produce spurious elevation of estimated magnesium values. Data in Table 4 indicate that recovery of added magnesium to urine is satisfactory.

DISCUSSION

The fluorometric estimation of magnesium in serum and urine as outlined by Schachter is dependent upon the observation that ethanolic solutions of an 8-hydroxyquinoline complex of magnesium emit a characteristic fluorescence when activated by light of wave length at 420 mμ with a peak intensity of fluorescence at 530 mμ.[23] This fluorescence of magnesium hydroxyquinoline is minimal from pH 3.5 to 5.0, but increases rapidly from pH 5.0 to 6.5. The increment in fluorescence from pH 3.5 to 6.5 is directly proportional to the magnesium concentration in serum and, with adjustment, to the magnesium concentration in urine. Other materials normally present exhibit a low fluorescence not dependent upon pH in this range.

Schachter tested a number of cations for their capacity to yield fluorescence in ethanol when treated with 8-hydroxyquinoline.[23] Only zinc demonstrated a significant increment in fluorescence. In the present study, the effect of various cations, under the pH range used for the determination of magnesium, produced only slight increase in fluorescence and a negligible increase in the estimated value of magnesium. Confirming Schachter's observations, only zinc produced sufficient interference in serum and urine to obviate a measurement of magnesium.[27, 29] The amounts of zinc added in our experiments, however, are far in excess of the normal concentration in serum and urine and, therefore, the contribution of zinc to the fluorescence in clinical situations may be regarded as negligible. This is particularly true inasmuch as the zinc concentration in human serum and urine is only approximately 2 to 3 per cent of the magnesium concentration.[37, 38]

There is favorable correlation of the normal range and mean values of serum magnesium as estimated by means of this fluorometric method and the values obtained by other, more widely used, technics (Table 1).

To our knowledge, there have been no previous reports of fluorometric estimations of magnesium in normal urines. In fact, there seems to be a paucity of information pertaining to the subject of urine magnesium. Many of the values in Table 5 appear in their sources without information as to the number of normals, methods used, or dietary influences upon the excretion. The reports of Thorén,[36] Walker and Walker,[42] and Heaton and Pyrah[19] are the best documented. The relatively wide range of normal urine magnesium in 24 hr. suggests that knowledge and control of dietary magnesium is important for the interpretation of renal conservation and excretion of the ion.

TABLE 5

NORMAL URINE MAGNESIUM

Source	Mean	Range
	mEq./24 hr.	
Aikawa[1]		5 to 17
British Medical Journal[3]		10 to 20
Danowski[9]	8.4	
Eastham[12]		14 to 24
Hänze[15]		6 to 12
Heaton and Pyrah[19]	9.0	5 to 13
Leichsenring and associates[24]	7.9*	
Sunderman and Boerner[35]	8.0	
Thorén[36]	5.6	1.4 to 9.8
Wacker and Vallee[41]	14.0	9.8 to 17.0
Walker and Walker[42]	9.0	2.2 to 25.6
This report	5.0	1.35 to 14.0

* All persons were women of college age.

SUMMARY

The property of fluorescence of ethanolic solutions of magnesium 8-hydroxyquinoline under appropriate light activation and pH is the basis for a sensitive, relatively specific, accurate, and rapid method for the estimation of magnesium in serum and urine. Interference from other cations and compounds, under standard conditions, is negligible. Mean normal values of 1.88 mEq. per l. in serum from 113 apparently normal people and 5.00 mEq./24 hr. in urine from 46 apparently normal people compare favorably with estimations of magnesium obtained by means of technics other than fluorometric measurement.

Fluorometric estimation of magnesium in serum and urine is recommended for clinical laboratory use. The value of *urine magnesium* as a diagnostic aid is limited by the wide range in normal persons and, therefore, dietary control and balance studies are recommended for diagnostic reliability.

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MAGNESIUM: THE EFFECTS OF INTRAVENOUS INJECTIONS ON THE HUMAN HEART*

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VERY little work is available concerning the parenteral effects of magnesium on the heart. However, it is not uncommon to run across such statements in the literature that "sudden death following the injection of a magnesium salt . . . is not an uncommon occurrence."[1] The literature has recently been summed up by Miller and Van Dellen, who in animal experiments found deleterious effects by magnesium on the cardiac conduction system.[1] The doses they used, however, were massive. In view of the increasing use of magnesium in the treatment of various types of cardiac disease,[2] and even more so in view of the great importance magnesium has assumed recently as a circulation time agent,[3, 4] we thought it apropos to determine in a systematic study the intravenous effects of *therapeutic* doses of magnesium on the human heart.

METHOD

One hundred adult patients were used in this investigation. One group of 34 cases was apparently free of cardiovascular disease; another group of 66 cases presented various forms and degrees of cardiac disease as outlined below. A total of 104 sets of electrocardiographic tracings were taken—69 in the cardiac group and 35 in the noncardiac group.

The three standard leads of the electrocardiogram were taken first. The electrocardiographic circuit was completed again, and simultaneously with the taking of Lead I, 10 c.c. of a warmed 10 per cent aqueous solution of magnesium sulfate were injected into a large antecubital vein of the reclining patient. In accordance with the technique of the magnesium circulation-time test,[4] the first 6 c.c. were injected through a No. 18 gauge needle as rapidly as possible (requir-

*From the Medical Service of Dr. Mitchell Bernstein, Jewish Hospital, Philadelphia.
Presented before the Jefferson Society for Clinical Investigation, Jefferson Medical College, May 18, 1939, Philadelphia.
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TABLE I

ILLUSTRATIVE ELECTROCARDIOGRAPHIC EFFECTS OF INTRAVENOUS MAGNESIUM SULFATE INJECTIONS

CASE	NAME	SEX	AGE	BLOOD PRESSURE		TRACING I DEPARTURES FROM NORMAL	TRACING II CHANGES DURING MAGNESIUM INJECTION	TRACING III CHANGES ONE HOUR AFTER INJECTION	REMARKS
				SYST.	DIAST.				
112	A. L.	M	50	104	60	None	T_3 raised (was flat)	QRS_1 decreased amplitude; T_3 raised (was flat); QRS_3 increased amplitude	Noncardiac patient Duodenal ulcer
56	A. F.	F	61	160	78	P waves low; QRS slightly slurred W QRS_3	QRS_2 moderately increased amplitude; W QRS_3 more marked	None	Arteriosclerotic heart disease
70	M. G.	M	63	126	40	Right axis deviation; QRS slurred and widened to 0.12 second; S_1 pronounced in all tracings; T_1 inverted	Absence of S_2; R_2 amplitude markedly increased	Absence of S_2; R_2 amplitude markedly increased	Acute coronary thrombosis; heart block (2:1 to 3:1) delayed conduction; arteriosclerotic heart disease
172	M. S.	M	67	176	78	QRS slurred; QRS_2 W shaped and negative; $T_{2,3}$ inverted; T_1 flat	T_2 flat (higher); QRS_2 became positive and lost the W type	T_2 flat (higher); QRS_2 became positive and lost W	Hypertensive heart disease; angina pectoris
53	B. E.	F	50	230	120	Left axis deviation; QRS slightly slurred; Q_3 deep; T_3 inverted	QRS M shaped and more slurred	None	Hypertensive heart disease
8	M. A.	F	54	240	140	QRS slurred; $T_{1,2}$ diphasic; left axis deviation	None	QRS_2 markedly decreased amplitude; QRS_3 increased amplitude	Old posterior coronary thrombosis; hypertensive heart disease
221	R. N.	M	19	94	60	Complete heart block (11/9/37); auricular rate 83, ventricular rate 30; QRS prolonged to 0.18 and slurred; T_1 inverted	None	QRS_3 narrower and higher (deeper)	Postscarlatinal complete heart block; three previous sets of tracings showed no changes

Tracing I—Set of three standard leads taken before injection of magnesium sulfate
Tracing II—Set of three standard leads taken during injection of magnesium sulfate
Tracing III—Set of three standard leads taken one hour after injection of magnesium sulfate

ing between one and one-half and two seconds), and the balance was injected more slowly. In a few cases 20 c.c. instead of 10 c.c. were injected. No injection required more than ten seconds, and most injections required considerably less. In succession, Leads II and III were then recorded, the total time elapsing from the moment of injection until the completion of Lead III being approximately three minutes. The time was spaced equally between the three leads. One hour later the standard leads were again recorded. Three sets of tracings were thus available for each case: a control set, a set taken during and closely after the injection, and a set one hour after the injection. These three sets are hereafter referred to as tracing I, tracing II, and tracing III, respectively. Space does not permit the publication of all of the available data, but illustrative cases are herein presented.

Noncardiac Group.—The group of noncardiac patients comprised 34 cases suffering from a wide assortment of diseases. It included 18 females and 16 males, ranging in age from 19 to 73 years. All were free of cardiovascular disease as ascertained by history, physical examination, and electrocardiographic evidence. Thirty-five sets of tracings were taken.

Ten patients showed electrocardiographic changes. Four patients showed delayed effects (that is, in tracing III); six patients developed changes both during the injection period and one hour later. No typical electrocardiographic pattern was observed. During the injection period Lead I was affected in only one case (Case 209) which showed a slight decrease in amplitude T_1. In one case (Case 61) T_3 was slightly decreased in amplitude; the reverse held true in another case (Case 112). The other changes consisted in very slight variations in amplitude of QRS_2 and QRS_3. The development in one case (Case 79) during the injection period of occasional U waves in Lead II, which subsequently disappeared, was interesting.

At no time were changes noted in the P waves, the duration of the P-R interval, or the heart rate. Case 112, Table I, had two sets of tracings, of which one showed no changes, and the other minor T and QRS changes. All other cases showed usually one minor variation.

Cardiac Group.—The cardiac group comprised 66 patients, 35 females and 31 males, ranging in age from 16 to 75. Sixty-nine sets of tracings were taken. One patient (Case 221, Table I) had four sets of tracings.

Arteriosclerotic Heart Disease.—Twenty-eight patients fell in the arteriosclerotic heart disease group; 9 had coexistent diabetes mellitus, of whom 2 had developed acute anterior coronary thrombosis; 3 had healed posterior coronary thrombosis; 2 had partial heart block (one complicated by an acute coronary thrombosis); one had an acute posterior coronary thrombosis; and 3 had auricular fibrillation. Two cases were complicated by pulmonary emphysema (one with bronchial asthma).

Of 10 patients in the arteriosclerotic heart disease group presenting electrocardiographic changes, only 2 showed changes during the injection period alone; 2 showed changes both during the injection and one hour later, and the balance showed deferred changes only. During the injection period the changes

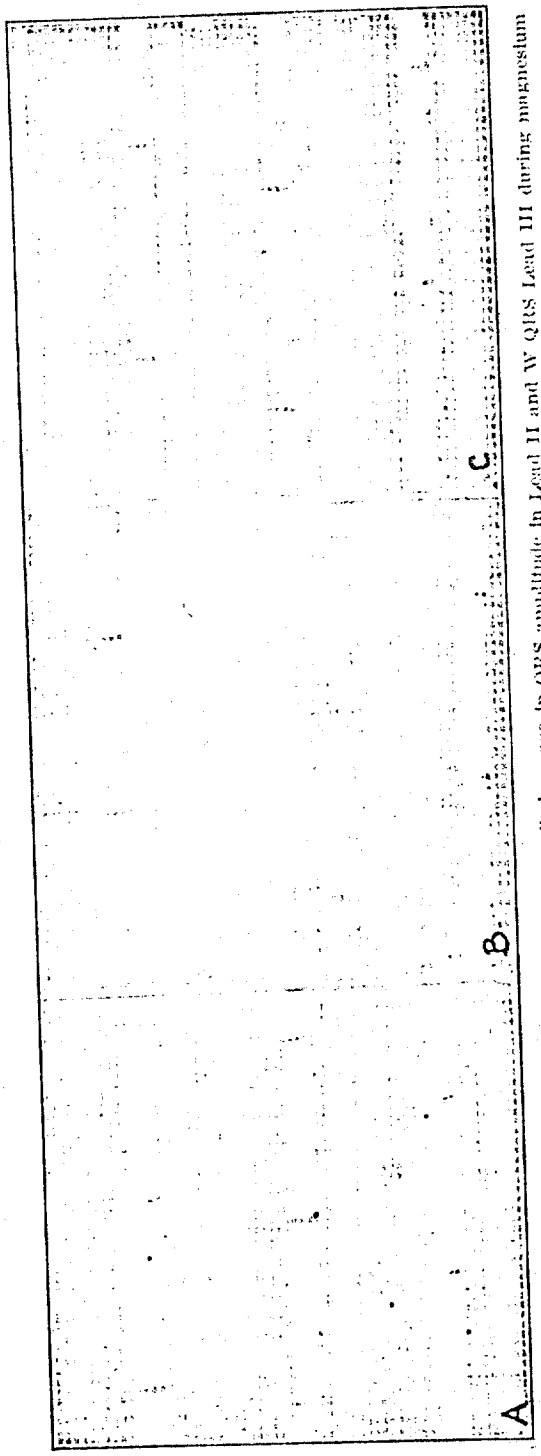

Fig. 1.—Case 56. *A*, Control before magnesium injection. *B*, Increase in QRS amplitude in Lead II and W QRS Lead III during magnesium injection. *C*, No change one hour after magnesium injection.

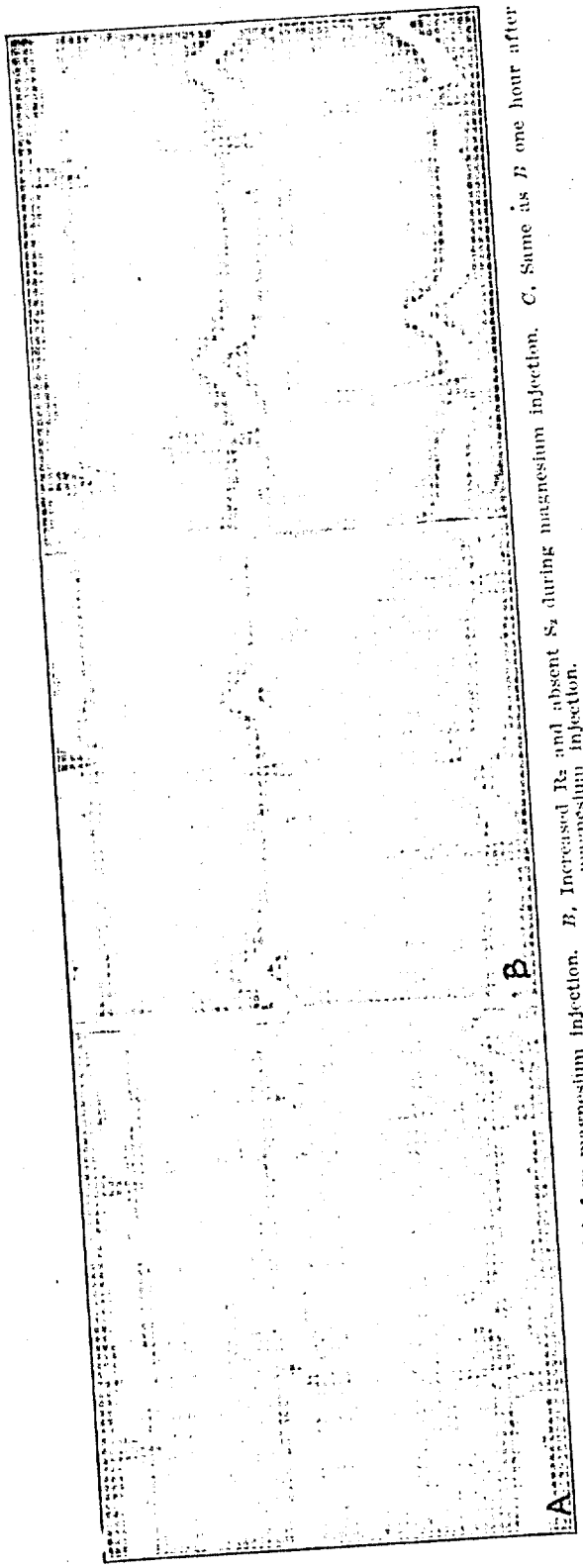

Fig. 2.—Case 70. *A*, Control before magnesium injection. *B*, Increased Rs and absent Ss during magnesium injection. *C*, Same as *B* one hour after magnesium injection.

were limited to increase of QRS_2 amplitude and accentuation of W QRS (Case 56, Fig. 1), and increase in amplitude of T_3 (Case 5). Case 70, shown in Fig. 2, presented a marked increase in R_2 amplitude as well as an absence of S_2 in both tracings II and III. One case (Case 3) developed a slight increase in T_2 amplitude in tracings II and III. In the deferred change group (tracing III) were decrease of QRS_3 amplitude (Case 49) and an interesting case (Case 168) in which QRS_3 changed from slurred to W. In one case (Case 13) QRS became lowered in amplitude and W shaped; in another case (Case 64) QRS_2 became lower in amplitude with marked slurring. The left axis deviation present in one case became more marked (Case 72). T_2 became inverted in one case (Case 102).

Hypertensive Cardiovascular Heart Disease.—The hypertensive cardiovascular disease group comprised 25 patients; 3 had coexistent diabetes mellitus (one with partial heart block), one had central nervous system syphilis, one hyperthyroidism, one angina pectoris, one had chronic glomerulonephritis, one auricular fibrillation, one a recent anterior coronary thrombosis, one an old posterior coronary thrombosis, and one an acute anterior coronary thrombosis.

Eleven patients in the hypertensive group presented electrocardiographic changes. Two developed changes during the injection period, 6 showed changes as deferred effects and 3 showed changes during the injection period and one hour later. During the injection period one patient (Case 35) developed an increase in amplitude of QRS_1; in another case (Case 53, Fig. 3) the QRS_2 complexes became M shaped with increased slurring. One case (No. 31) in which QRS_3 was inverted and W shaped became positive in tracing II and showed a mixture of positive and negative complexes in tracing III. Case 65 showed an increase in QRS_2 amplitude with increased slurring in tracing II, an effect which disappeared in tracing III, only to be replaced by deepening of the T_1 and T_2 waves. Another interesting case (Case 172, Fig. 4) displayed an increase of T_2 amplitude in tracings II and III as well as QRS_2 becoming positive and losing the W. As deferred effects (tracing III) were: QRS showed slightly decreased amplitude in one case (Case 37); QRS_2 decreased and QRS_3 increased in amplitude in one case (Case 8, Fig. 5); QRS_1 amplitude increased and the QRS_3 complex became inverted in another case (Case 69); QRS_3 changed from negative to positive (Case 171); QRS_1 showed slightly increased slurring in one case (Case 212) and the T waves increased slightly in voltage in another case (Case 84).

Miscellaneous Cases of Cardiac Disease.—Of a miscellaneous group comprising 8 patients there were 2 with chronic rheumatic heart disease, one with a post-scarlatinal complete heart block, one with a syphilitic dissecting aneurysm of the arch of the aorta, 2 with congenital heart disease (the heart of one was complicated by a subacute bacterial pulmonary arteritis), one with hyperthyroidism and one with a subacute bacterial endocarditis; two patients showed electrocardiographic changes during the injection period and one patient showed changes as a delayed effect. One of the foregoing cases (Case 188) developed inversion of T_1 and T_2, another (Case 145) an increase of R_3 amplitude during the injection period, and a third (Case 60) a marked decrease of R_2 amplitude as a

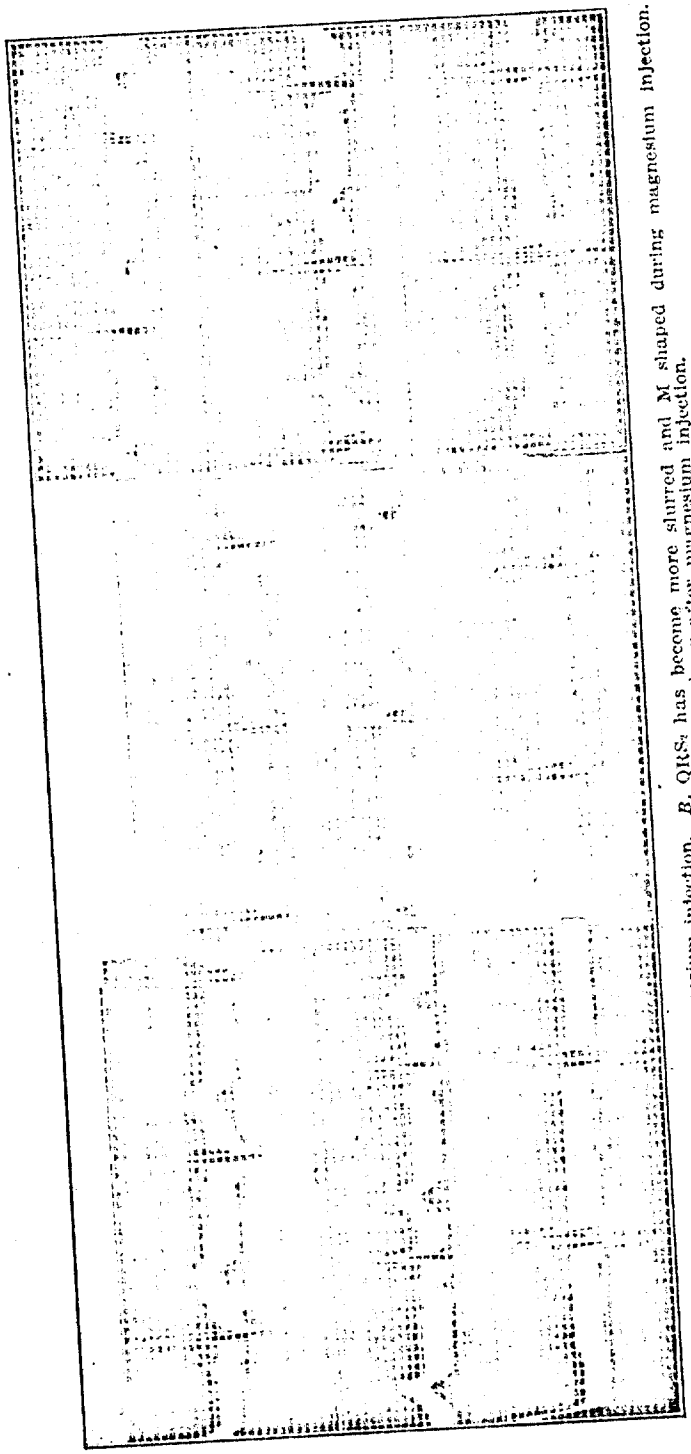

Fig. 3.—Case 53. *A*, Control before magnesium injection. *B*, QRS$_2$ has become more slurred and M shaped during magnesium injection. *C*, Showing reversion to control one hour after magnesium injection.

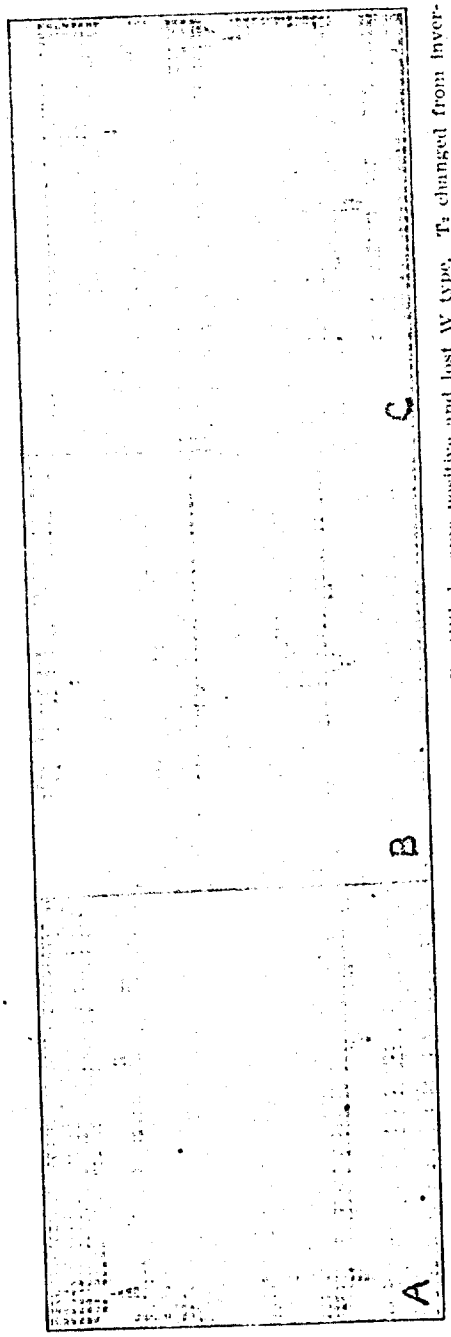

Fig. 4.—Case 172. *A*, Control before magnesium injection. *B*, QRS₂ became positive and lost W type. T₂ changed from inversion to flat during magnesium injection. *C*, Same changes as noted in *B* one hour after injection.

layed effect. A case of complete heart block (Case 221)[9] had four sets of tracings, of which the first three showed no changes, and the fourth a narrowing and deepening of the QRS_3 complex one hour after the injection.

Equivocal Heart Disease.—In a group of 5 young adults with electrocardiographic changes as the only evidence of cardiac disease, no changes developed during the injection period. One patient (Case 126) developed a sinus arrhythmia and one patient (Case 113) a slightly deeper Q_3 wave in tracing III.

In résumé, the cardiac group as a whole showed slight electrocardiographic changes in 26 of the 66 cases. Only 6 patients were limited to the injection period, 5 to effects both during the injection and subsequently, and 15 patients to the deferred period only. An outstanding feature is that the changes were confined almost exclusively to the T waves and QRS complexes. Even more noteworthy is the fact that during the true injection period (shown only in Lead I of tracing II), only two minor changes were recorded in the entire series. There were no P wave changes, no changes in the P-R interval or in the heart rate. No ventricular premature beats were noted. There were no bradycardias or cases of sinus arrest. Eleven patients who were receiving digitalis at the time of the injections, showed no untoward effects.

COMMENT

The striking feature is the complete lack of uniformity of changes wrought upon the heart by magnesium. That magnesium may have a direct effect upon the myocardium of the ventricles is indicated by the slight changes in QRS complexes and T waves occurring in a fairly high percentage of cases. The types of changes produced during the injection period and one hour later are practically the same, whether in the cardiac or noncardiac groups. That these changes are negligible is shown by the fact that Lead I (taken during the injection when the full brunt of the magnesium was borne) showed only one slight T_1 change in the noncardiac group and only one slight QRS_1 and one T_1 change in 2 of the cardiac cases. Moreover, very few patients showed more than one lead change whether during the injection or one hour afterwards. In 2 patients, one a noncardiac person and the other a critically ill complete heart block individual, in whom two sets and four sets of tracings were obtained respectively, it is noteworthy that minor changes were obtained in only one set of tracings in each patient. These results illustrate the inconstancy of magnesium effect. In general, there were as many changes in T waves and QRS complexes in one direction as there were in the opposite. Finally, such changes as did occur were evanescent inasmuch as standard electrocardiograms, taken in the vast majority of the patients within a few days after the injection, showed all magnesium effects to have vanished entirely. Consequently, we must conclude that the rapid intravenous injection of 10 per cent magnesium sulfate exerts no deleterious action on the heart.

It is interesting to compare these results with the cardiac effects induced by the intravenous use of 20 per cent calcium gluconate, a substance that has come into wide use recently as a circulation-time reagent. In normal individuals

calcium produces flattening or inversion of the T waves in 92 per cent of cases, flattening or inversion of the P waves in 54 per cent, and a marked bradycardia in 67 per cent.[5] No systematic studies of the cardiac effects of intravenous calcium injections in cardiac patients are available, so that we have no basis for direct comparison of the effects of magnesium and calcium in cardiac disease. That calcium injections clinically may lead to catastrophes in both normal and diseased hearts is now well substantiated.[5-8]

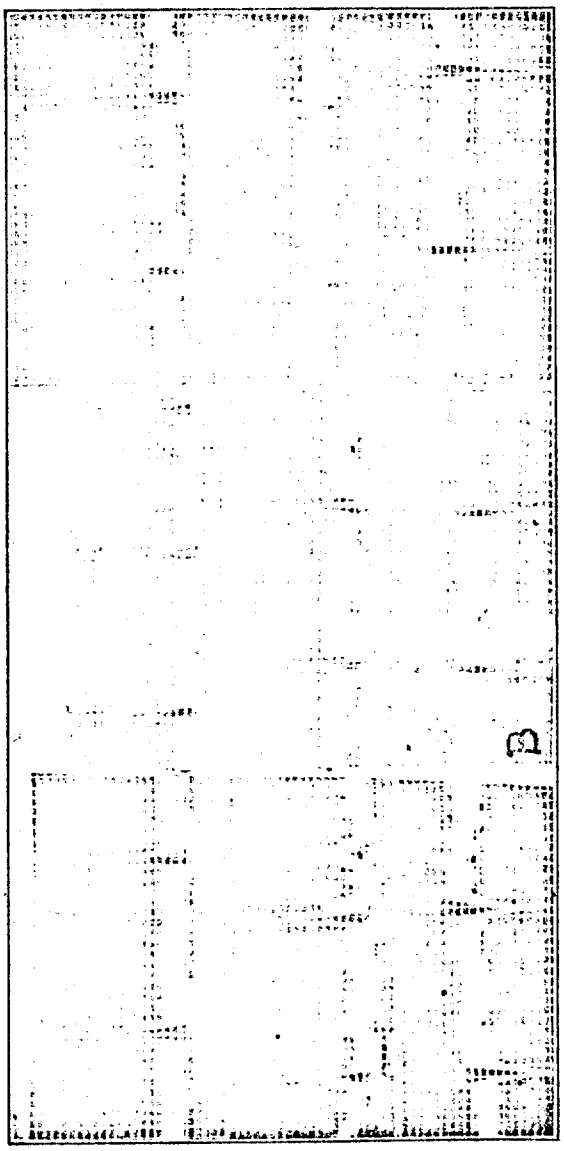

FIG. 5.—Case 8. *A*, Control before magnesium injection. *B*, QRS$_2$ lower voltage and QRS$_3$ increased voltage during magnesium injection. *C*, QRS$_2$ depressed and QRS$_3$ increased one hour after magnesium injection.

SUMMARY

1. The effects of the rapid intravenous injection of 10 c.c. of 10 per cent magnesium sulfate on the human heart were recorded electrocardiographically in 100 patients. The three standard leads of the electrocardiogram were re-

corded prior to injection, simultaneously with the injection, and one hour later. Thirty-five sets of tracings were taken in 34 noncardiac patients and 69 sets in 66 cardiac patients.

2. The cardiac group included 28 persons with arteriosclerotic heart disease, 25 persons with hypertensive heart disease, 5 persons with only electrocardiographic evidence of cardiac disease, and a miscellaneous group of 8 cases comprising 2 persons with chronic rheumatic heart disease, one with postscarlatinal complete heart block, one with syphilitic dissecting aneurysm of the aortic arch, two with congenital heart disease (one complicated by a subacute bacterial pulmonary arteritis), one with subacute bacterial endocarditis, and one with hyperthyroidism.

3. In the noncardiac group 6 patients presented minor T and QRS changes (usually in one lead) both during the injection period and subsequently; 4 persons showed similar changes one hour after injection.

4. In the cardiac group similar changes were exhibited by 6 persons during the injection period, by 15 persons one hour after injection, and by 5 persons both during the injection period and one hour later.

5. A discussion of the changes produced leads to the conclusion that intravenous magnesium injections exert no deleterious effect on the human heart. The coincidental administration of digitalis yields no untoward effects.

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1321 SPRUCE STREET

FRENCH TRANSLATION

Microanalysis of Magnesium in Various Biological Mediums

by: C. Bohuon

Clin. Chim. Acta 7: 811-817 (1962)

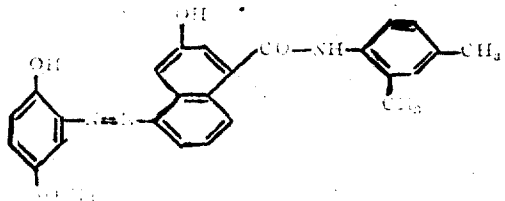
Recently a number of publications deal with the importance of magnesium in various pathological syndromes and its intervention in many enzymatic processes. They have brought fresh data on the physiopathology of this element. Unfortunately the many techniques for determining magnesium in biological media suggested up until now are either not very precise or tricky to carry out. The majority of the present techniques are based on the colorimetric measurement of the red lake produced by titanium yellow and magnesium in an alkaline medium or the use of complexometric titration with various indicators of which the most well known is black Eriochrome.

The techniques based on the use of titanium yellow (for example that of Garner or of Masson) are sufficiently precise, but recessitate the precipitation of proteins and a fairly rapid photometric reading.

Complexometric techniques are not very precise when serum is directly utilised. To obtain the greatest precision it is necessary to carry out the titration of magnesium in the form of ammonium magnesium phosphate, after the elimination of calcium as the oxalate.

We therefore thought that it would be worthwhile to put a technique at the disposition of biochemists which combines great simplicity with sensitivity. Our purpose also was to reduce as much as possible the amount of serum or cerebrospinal fluid needed for the determination. Our choice therefore recessitated proceeding with a highly sensitive reaction. In fact, in 1956 these authors discovered a new magnesium reagent: the sodium

salt of 1-azo-2-hydroxy-3-(2,4-dimethylcarboxy)anilino)naphthalene-1-(2-hydroxybenzene-4-sulfonic acid), eight times more sensitive than titanium yellow and very specific.



At pH 9-10 this reagent is blue which a trace of magnesium in dilute alcohol turns to pink.

After a number of experiments designed to eliminate the precipitation and interference of proteins in the alcoholic medium, we adopted the technique described below. It is only necessary to use a sample of 100 µl of plasma or cerebrospinal fluid. But smaller quantities can be easily used, which makes it permissible to classify this among the ultra micromethods.

Suggested Technique

Materials:

A 25 ml volumetric flask preferably with a ground glass stopper; spectrophotometer Unicam or Jobin and Yvon Moroccan standard etc. 30 mm or better 40 mm cells.

Reagents

(1) Mann and Yoe* reagent: Dissolve 25 mg of reagent in 200 ml of absolute alcohol R.P. with refluxing. When dissolved make up to 250 ml with absolute alcohol, carefully.

(2) 0.08 M sodium borate: Dissolve 30.51 g sodium borate R.P. in the cold in 500 ml of double distilled water. Make up to 1000 ml with double distilled water, carefully.

*Manufactured by LaMotte Chemical Products Co., Chestertown, Md., U.S.A.

(3) Absolute ethyl alcohol.

(4) Concentrated magnesium solution: 1 ml = 100 µg. Weigh 1.0131 g of magnesium sulfate R.P.:$MgSO_4 \cdot 7H_2O$. Dissolve in a 1000 ml volumetric flask in double distilled water, then make-up to the line on the flask. Keep cold.

(5) Dilute magnesium soltion: 1 ml = 1 µg, by dilution to 1/100th of the mother solution.

Method

In a 25 ml graduated flask, place 9.9 ml of double distilled water; 0.1 ml of serum or plasma carefully rinsing the pipette; 5 ml of reagent 1, shake; 2 ml of reagent 2, shake; make up to 25 ml with absolute alcohol.

The color develops in a few minutes and sometimes shows up after a 20 minutes wait. It is stable for 24 hours. The reading is made at 5050 Å in 30 or 40 mm cells by comparison with a blank reagent (or against water). It is necessary to make a standard scale for each new preparation of the Mann and Yoe reagent.

Table 1

Standard Scale for the Reagent

	Preparation (ml)	Blank (ml)	1	2	3	4	5	6	7
Dilute sol. 1 ml = 1 µg			1	2	3	4	5	6	7
Double distilled water	9.9	10	9	8	7	6	5	4	3
Serum or plasma	0.1								
Mann and Yoe Reagent	5	5	5	5	5	5	5	5	5
Sodium borate 0.08 M	2	2	2	2	2	2	2	2	2
Absolute alcohol q.s.p.	25	25	25	25	25	25	25	25	25

Calculation

For the calculation refer to the standard curve (Fig. 1). The magnesium content in mg per liter is given by the formula:

Value read on the curve in µg x 10,000 = magnesium in mg/l of serum or plasma.

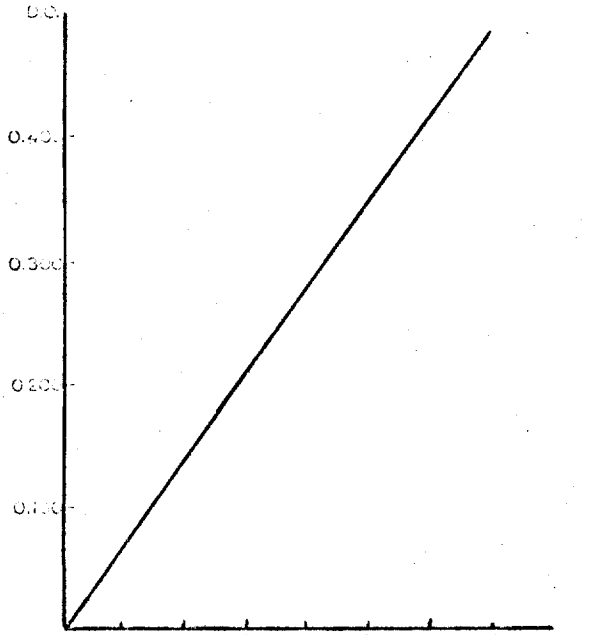


Fig. 1.

The normal amount of Mg found by this technique in plasma or serum is 22 mg ± 3.

Critical study of the Technique

Choice of wavelength

We have studied the absorption spectra in the ultraviolet and the visible of the reagent with and without magnesium (Fig. 2). We can see that the spectra are very different in these two cases.

Study on the evolution of the coloring

The coloring develops very rapidly, in about 5 minutes, and it is stable for 24 hours at laboratory temperature. This is a very great advantage over most of the other techniques.

Table 2

Quantity added (µg)	Quantity recovered	Percentage recovered	Quantity added (µg)	Quantity recovered	Percentage recovered	Quantity added (µg)	Quantity recovered	Percentage recovered
1	1.05	105	2	1.95	96.5	3	3	100
1	0.93	93	2	1.90	95	3	3	100
1	1.06	106	2	2.07	103	3	3.05	101
1	1.03	103	2	2	100	3	3.05	101
1	0.98	98	2	2.05	101	3	2.9	97.5
			2	2	100	3	3.02	100
			2	2	100			

Table 3

Sample No.	Optical density	Magnesium in mg/l
1	0.280	38.4 m
2	0.280	38.4 m
3	0.280	38.4 m
4	0.282	38.5 m
5	0.278	38.3 m

Calcium interference

Calcium interference is one of the principal problems in complexometric techniques. But we have put overloads of 20 µg of calcium in the preparations and in the range (from 1 to 8 times the quantity of magnesium present) we have not noted any interference.

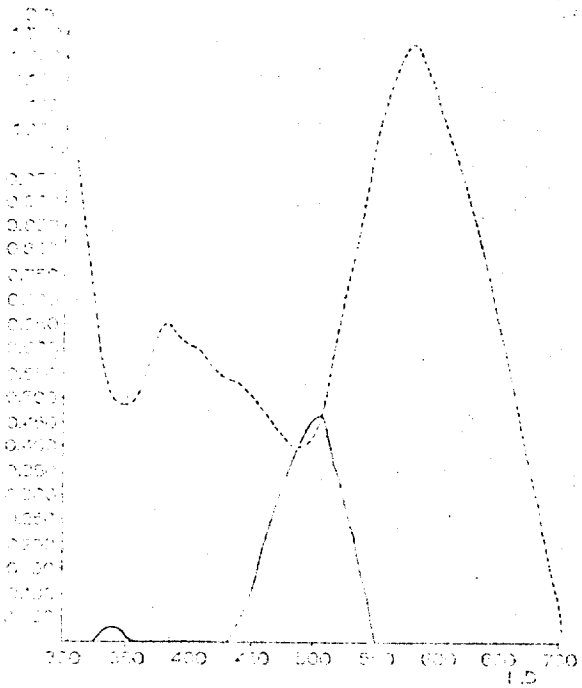


Fig. 2.

Magnesium overload

To verify the accuracy of the suggested technique, we loaded a number of plasmas samples with increasing quantities of magnesium. The results are detailed in Table 2.

The amounts added were therefore recovered with an average value of near 100%, these excellent results can undoubtedly be explained by the very reduced number of manipulations necessitated by this method.

Reproducibility, standard deviation

The reproducibility of results is remarkable and the errors are due solely to the pipetting and the photometric reading.

Here for example (Table 3) are the results gotten from repeating the determination on the same serum five times.

Adaptation of the technique of magnesium determination in urine and cerebrospinal fluid

(a) Cerebrospinal fluid. The determination of magnesium in cerebrospinal fluid is carried out exactly as for plasma.

(b) Urine. For determination in urine it is first necessary to acidify the urine with hydrochloric acid to pH 3-4. The urine is next diluted 1/10. In the case of normal urine 0.5 ml is taken. The sample size can be increased or lessened in the case of urine either very poor or very rich in magnesium. It is made up to 15 ml with double distilled water and the technique for determination in plasma is followed.

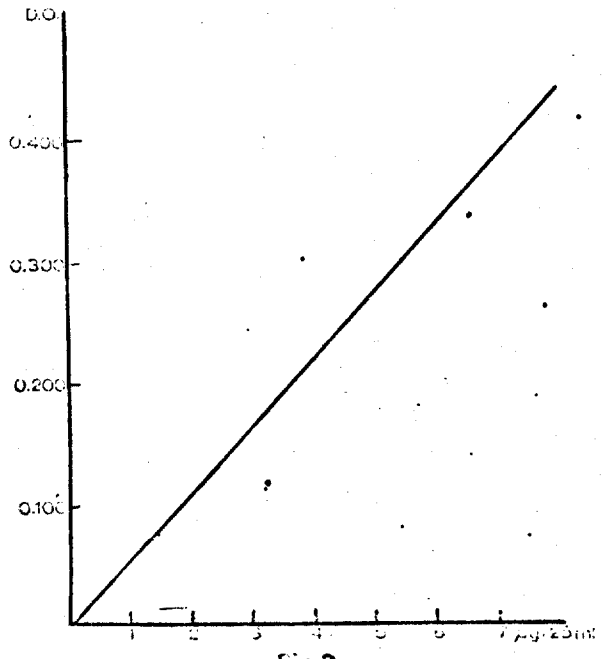


Fig. 3

Determination of magnesium in nephrotic or jaundiced serum

In certain serums encountered in exceptional cases: nephrosis or jaundice (bilirubinemia at 200 mg/l) direct determination is not possible and it is necessary to clarify the serum. We have adopted the tungstic clarification according to Folin and Wu which does not inhibit the coloration very much as is shown on Fig. 3 and which is therefore superior to trichloracetic acid clarification.

Method

Serum or plasma, 1 ml; 7 ml double distilled water; 1 ml of 10% sodium tungstate, (shake); 2/3 N H_2SO_4, 1 ml (shake).

Wait a few minutes and centrifuge. Make a blank by replacing the serum with double distilled water (Table 4). Take one ml of the clear supernatant and add successively: 7 ml of double distilled water; 5 ml of Mann and Yoe reagent and 4 ml of 0.08 M sodium borate. Make up to 25 ml with absolute alcohol.

Magnesium determination in red blood corpuscles

The blood is collected over heparin then centrifuged. Remove the plasma. Wash the corpuscles with physiological serum 3 times. Take 0.5 ml of the pure globules with a dry calibrated pipette. Let it gently run out into 7.5 ml of double distilled water. Rinse the pipette well by blowing and sucking several times in order to effect hemolysis. The rest of the procedure is the same as for nephrotic serum.

Results obtained

The normal values are as follows: plasma, from 19-25 mg/liter with an average of 22 mg. Red blood corpuscles, about 60 mg/1000; cerebrospinal fluid, 24 mg/l. Urines, quite variable, on average 50-100 mg/l.

Table 4

	Preparation	Blank	1 µg	2 µg	3 µg	4 µg	5 µg	6 µg	7 µg
Dilute solution of Mg^{2+}			1 ml	2	3	4	5	6	7
Clarified (determination)	1								
Clarified (blank)		1	1	1	1	1	1	1	1
Double distilled water	7	7	6	5	4	3	2	1	
Mann and Yoe reagent	5	5	5	5	5	5	5	5	5
0.08 M sodium borate	4	4	4	4	4	4	4	4	4
25 ml absolute alcohol q.s.p.									

Resume

We propose a very simple, rapid microtechnique (about one minute per determination), precise and adaptable to all the current biological mediums. It is also possible with this technique to easily establish magnesium distribution in infants. By reducing the sample it is also equally possible to use it for the determination of magnesium in little animals.

MICRODOSAGE DU MAGNÉSIUM DANS DIVERS MILIEUX BIOLOGIQUES

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(Reçu le 20 octobre, 1961)

INTRODUCTION

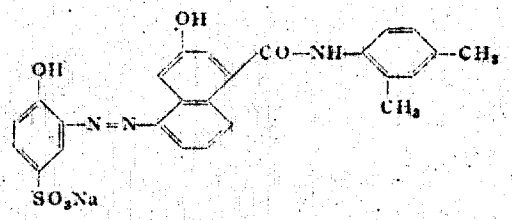
De nombreuses publications récentes traitent de l'importance du magnésium dans divers syndromes pathologiques et de son intervention dans beaucoup de processus enzymatiques. Elles ont apporté de nouvelles précisions sur la physiopathologie de cet élément[1-3]. Malheureusement les multiples techniques proposées jusqu'ici pour doser le magnésium dans les milieux biologiques sont soit peu précises, soit délicates à exécuter[4]. La plupart des techniques actuelles reposent sur la mesure colorimétrique de la laque rouge donnée par le jaune titane et le magnésium en milieu alcalin ou sur l'emploi titrimétrique des complexons avec des indicateurs variés dont le plus connu est le noir Eriochrome.

Les techniques fondées sur l'emploi du jaune titane (par exemple celle de GARNER[5] ou de MASSON[6]) sont assez précises, mais exigent une précipitation des protéines et une lecture photométrique assez rapide.

Les techniques complexométriques sont peu précises lorsque le sérum est utilisé directement. Pour obtenir une grande précision, il est nécessaire d'opérer la titration du magnésium sous forme de phosphate ammoniaco-magnésien, après élimination du calcium sous forme d'oxalate.

Nous avons donc pensé qu'il serait utile de mettre à la disposition des biochimistes une technique qui allie une grande simplicité à une bonne sensibilité. Notre but était aussi de réduire au maximum la quantité de sérum ou de liquide céphalo-rachidien nécessaire au dosage.

Notre choix devait donc se porter sur un réactif d'une haute sensibilité. Nous avons pensé utiliser le réactif de MANN ET YOE[7]. En effet, ces auteurs ont découvert en 1956 un nouveau réactif du magnésium: le 1-azo-2-hydroxy-3-(2,4-diméthylcarboxanilido)naphtalène-1(2-hydroxybenzène-4-sulfonate de sodium), huit fois plus sensible que le jaune titane et très spécifique.



À pH 9–10, ce réactif présente une couleur bleue que des traces de magnésium vont virer au rose en milieu hydroalcoolique.

* Adresse actuelle: Unité de Biologie Clinique, Institut Gustave Roussy, Villejuif (France).

Après de nombreux essais destinés à éviter la précipitation et l'interférence des protéines en milieu alcoolique, nous avons adopté la technique décrite ci-dessous. Elle ne nécessite pour être utilisée qu'une prise d'essai de 100 μl de plasma ou de liquide céphalo-rachidien. Mais on peut facilement utiliser des quantités moindres, ce qui permet donc de la classer parmi les ultra-microméthodes.

TECHNIQUE PROPOSÉE

Matériel

Fioles jaugées de 25 ml avec bouchage émeri de préférence; spectrophotomètre Unicam ou Jobin et Yvon type Maroc, etc.; cuves de 30 mm ou mieux de 40 mm.

Réactifs

1. Réactif de MANN ET YOE *: Dissoudre 25 mg du réactif dans 200 ml d'alcool absolu R.P. par ébullition à reflux. Après dissolution, compléter à 250 ml avec de l'alcool absolu. Bonne conservation.

2. Borate de sodium 0.08 *M*: Dissoudre 30.51 g de borate de sodium R.P. à chaud dans 500 ml d'eau bidistillée. Compléter à 1,000 ml avec de l'eau bidistillée. Bonne conservation.

3. Alcool éthylique absolu.

4. Solution concentrée de magnésium: 1 ml = 100 μg. Peser 1.0131 g de sulfate de magnésium R.P.: $MgSO_4 \cdot 7\ H_2O$. Dissoudre dans une fiole jaugée de 1,000 ml dans l'eau bidistillée, puis compléter au trait de jauge. Conserver en chambre froide.

5. Solution diluée de magnésium: 1 ml = 1 μg, par dilution au centième de la solution-mère.

Mode opératoire

Dans un ballon jaugé de 25 ml, introduire: 9.9 ml d'eau bidistillée; 0.1 ml de sérum ou plasma en rinçant soigneusement la pipette; 5 ml de réactif 1, agiter; 2 ml de réactif 2, agiter; compléter à 25 ml avec de l'alcool absolu.

La coloration se développe en quelques minutes et peut être lue après une attente de 20 min. Elle est stable pendant 24 h. La lecture se fait à 5050 Å en cuves de 30 ou 40 mm par comparaison avec un témoin réactif (ou contre l'eau). Il est nécessaire de faire pour chaque nouvelle préparation de réactif de MANN ET YOE une gamme-étalon.

TABLEAU I

GAMME ÉTALON DE RÉACTIF

	Dosage (ml)	*Témoin (ml)*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Sol. diluée 1 ml = 1 μg			1	2	3	4	5	6	7
Eau bidistillée	9.9	10	9	8	7	6	5	4	3
Sérum ou plasma	0.1								
Réactif de MANN ET YOE	5	5	5	5	5	5	5	5	5
Borate de sodium 0.08 *M*	2	2	2	2	2	2	2	2	2
Alcool absolu *q.s.p.*	25	25	25	25	25	25	25	25	25

* Fabriqué par LaMotte Chemical Products Co. Chestertown, Md. (USA).

Calcul

Pour les calculs se reporter à la courbe étalon (Fig. 1). La teneur du magnésium en mg par litre est donnée par la formule:
valeur lue sur la courbe en μg × 10,000 = magnésium en mg/l de sérum ou de plasma.

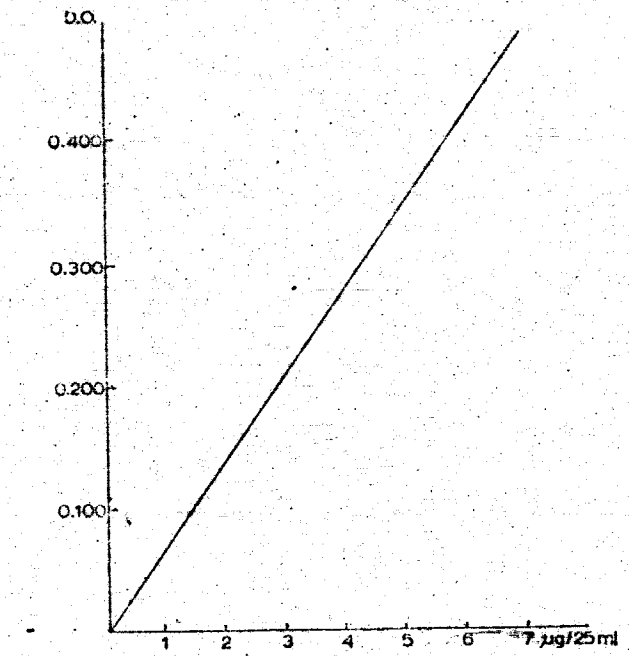


Fig. 1.

Les quantités normales de Mg trouvées par cette technique dans le plasma ou le sérum sont de 22 mg ± 3.

ÉTUDE CRITIQUE DE LA TECHNIQUE

Choix de la longueur d'onde

Nous avons étudié les spectres d'absorption dans l'ultraviolet et le visible du réactif avec et sans magnésium (Fig. 2). Nous pouvons observer que le spectre est très différent dans les deux cas.

Étude de l'évolution de la coloration

La coloration se développe très rapidement, en 5 min environ, et elle est stable à la température du laboratoire pendant 24 h. C'est un très gros avantage sur la plupart des autres techniques.

Interférence du calcium

L'interférence du calcium est une des principales difficultés des techniques complexométriques. Or nous avons fait des surcharges de 20 μg de calcium dans nos dosages et dans la gamme (soit de 1 à 8 fois la quantité du magnésium présent) nous n'avons constaté aucune interférence.

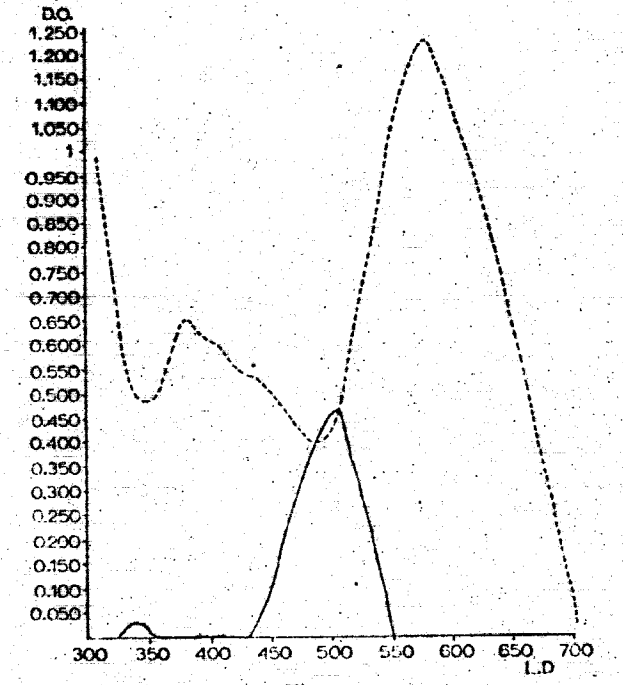


Fig. 2.

Surcharges en magnésium

Pour vérifier l'exactitude de la technique proposée, nous avons surchargé de nombreux échantillons de plasma avec des quantités croissantes de magnésium; les résultats sont détaillés dans le Tableau II.

TABLEAU II

Quantité ajoutée (µg)	Quantité retrouvée	Pourcentage récupéré	Quantité ajoutée (µg)	Quantité retrouvée	Pourcentage récupéré	Quantité ajoutée (µg)	Quantité retrouvée	Pourcentage récupéré
1	1.05	105	2	1.95	96.5	3	3	100
1	0.93	93	2	1.90	95	3	3	100
1	1.06	106	2	2.07	103	3	3.05	101
1	1.03	103	2	2	100	3	3.05	101
1	0.98	98	2	2.05	101	3	2.9	97.5
			2	2	100	3	3.02	100
			2	2	100			

TABLEAU III

Echantillon No.	Densité optique	Magnésium en mg/l
1	0.280	38.4 m
2	0.280	38.4 m
3	0.280	38.4 m
4	0.282	38.5 m
5	0.278	38.3 m

Les quantités ajoutées sont donc retrouvées avec une évaluation moyenne voisine de 100%; ces excellents résultats s'expliquent sans doute par le nombre très réduit des manipulations exigées par cette méthode.

Reproductibilité, écart standard

La reproductibilité des résultats est remarquable et les erreurs sont dues uniquement aux pipetages et aux lectures photométriques.

Voici par exemple les résultats obtenus en répétant le dosage 5 fois sur le même sérum (Tableau III).

Adaptation de la technique de dosage du magnésium dans l'urine et le liquide céphalo-rachidien

Liquide céphalo-rachidien. Pour doser le magnésium dans le liquide céphalo-rachidien, il suffit d'opérer exactement comme pour le plasma.

Urine. Pour le dosage dans l'urine il est nécessaire d'abord d'acidifier l'urine à pH 3–4 avec de l'acide chlorhydrique. L'urine est ensuite diluée 1/10. On en prélève 0.5 ml dans le cas d'urines normales. La prise d'essai peut être augmentée ou diminuée dans le cas d'urines très pauvres ou très riches en magnésium. On complète à 5 ml avec de l'eau bidistillée et l'on poursuit la technique comme pour le dosage dans le plasma.

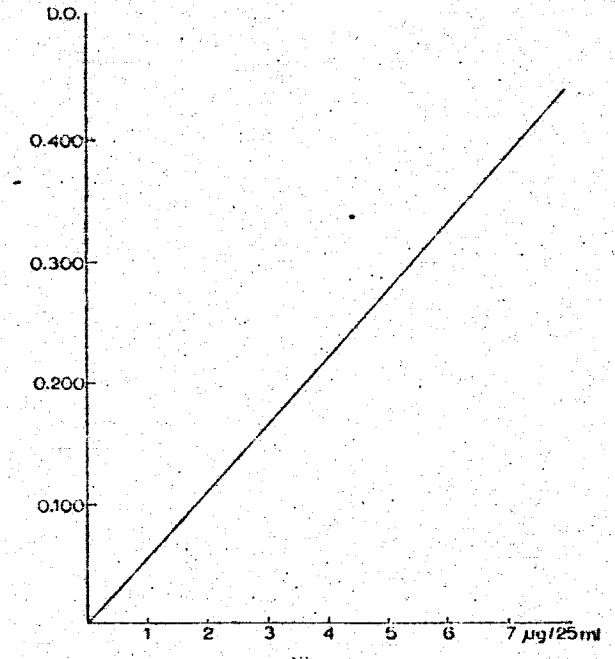


Fig. 3.

Dosage du magnésium dans les sérums néphrotiques et ictériques

Dans certains sérums rencontrés exceptionnellement: néphroses ou ictères (bilirubinémie à 200 mg/l) le dosage direct n'est plus possible et il est nécessaire de défé[quer] le sérum. Nous avons adopté la défécation tungstique selon Folin et Wu qu

inhibe peu la coloration comme le montre la Fig. 3 et qui est ainsi supérieure à la défécation trichloracétique.

Mode opératoire: Sérum ou plasma, 1 ml; eau bidistillée, 7 ml; tungstate de sodium à 10%, 1 ml (agiter); H_2SO_4N 2/3, 1 ml (agiter).

Attendre quelques minutes et centrifuger. Faire un témoin en remplaçant le sérum par de l'eau bidistillée (Tableau IV). Reprendre 1 ml du liquide surnageant

TABLEAU IV

	Dosage	*Témoin*	*1 µg*	*2 µg*	*3 µg*	*4 µg*	*5 µg*	*6 µg*	*7 µg*
Solution diluée de Mg^{2+}			1 ml	2	3	4	5	6	7
Déféqué (dosage)	1								
Déféqué (témoin)		1	1	1	1	1	1	1	1
Eau bidistillée	7	7	6	5	4	3	2	1	
Réactif de MANN ET YOE	5	5	5	5	5	5	5	5	5
Borate de Na 0.08 *M*	4	4	4	4	4	4	4	4	4
Alcool absolu *q.s.p.* 25 ml									

clair et ajouter successivement: 7 ml d'eau bidistillée; 5 ml du réactif de MANN ET YOE et 4 ml de borate de sodium 0.08 *M*. Compléter à 25 ml avec de l'alcool absolu.

Dosage du magnésium dans les globules rouges

Le sang est recueilli sur héparine puis centrifugé. Éliminer le plasma. Laver 3 fois les globules avec du sérum physiologique. Avec une pipette jaugée à sec, prendre 0.5 ml de la purée globulaire. Laisser écouler doucement dans 7.5 ml d'eau bidistillée. Bien rincer la pipette en soufflant et aspirant plusieurs fois afin de réaliser l'hémolyse. Les opérations suivantes sont les mêmes que dans les sérums néphrotiques.

RÉSULTATS OBTENUS

Les valeurs normales sont les suivantes: Plasma, de 19 à 25 mg/l avec une moyenne de 22 mg. Globules rouges, environ 60 mg/1,000. Liquide céphalo-rachidien, 24 mg/l. Urines, assez variable, en moyenne 50 à 100 mg/l.

REMERCIEMENTS

Nous remercions Monsieur le Doyen RENÉ FABRE, Pharmacien des Hôpitaux, pour les conseils qu'il nous a donnés au cours de ce travail. Nous remercions également le docteur RAYNAUD qui nous a fait part de ses observations au sujet de ce travail.

RÉSUMÉ

Nous proposons une microtechnique très simple, rapide (une minute environ par dosage), précise et adaptable à tous les milieux biologiques courants. Il est possible avec cette technique d'établir des bilans magnésiens avec une grande facilité, même chez les nourrissons. En réduisant la prise d'essai, elle peut également être utilisée pour doser le magnésium chez les petits animaux.

SUMMARY

MICRODETERMINATION OF MAGNESIUM IN BIOLOGICAL FLUIDS

A simple, precise and rapid micromethod for the estimation of magnesium in some

biological fluids has been described. The use of this method makes possible and easy the studies of magnesium metabolism in babies or little animals (mouse or rat).

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MAGNESIUM SULFATE IN PAROXYSMAL TACHYCARDIA*

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Although the cardio-inhibitory action of magnesium salts has been known for a long time,[1,2,3] it is rarely employed for therapeutic purposes in cardiac disease. Beneficial effects should be expected in disturbances resulting from augmented myocardial irritability or increased stimulus formation.

In 1930, Seekles employed magnesium chloride intravenously to prevent arrhythmias evoked by intravenous injections of calcium chloride, given to cows suffering from milk fever or grass staggers (grass tetany). The combination of magnesium chloride and calcium chloride yielded better results. In 1935 Zwillinger recommended magnesium sulfate by vein for the treatment of premature contractions and paroxysmal tachycardias. A patient lost consciousness and appeared dead when many multifocal extrasystoles and ventricular flutter occurred after the intravenous injection of 0.25 mg. of strophanthin; an intracardiac injection of 10 cc. of a 15% solution of magnesium sulfate revived him, the flutter subsiding and the extrasystoles vanishing for a short time. On the basis of this experience the author employed 10 to 15 cc. of a 15% solution of magnesium sulfate, in 1 instance even 10 cc. of a 30% solution, in patients suffering from paroxysmal auricular tachycardia; in all cases the tachycardia imme-

* Aided by a Grant from the Council on Pharmacy and Chemistry, The American Medical Association.

diately stopped and sinus rhythm was restored. No effect was obtained with this remedy in 3 cases of auricular flutter and 2 of auricular fibrillation.

Medical literature is relatively silent on the therapeutic effect of magnesium salts on paroxysmal tachycardia. On the basis of animal experiments, Smith, Winkler and Holf state that "it seems rather doubtful whether the small doses recommended have much effect on cardiac rhythm." The present paper reviews our experience with 11 patients treated by an intravenous injection of magnesium sulfate.

TABLE 1.—RESULTS OF INTRAVENOUS INJECTIONS OF MAGNESIUM SULFATE

No.	Name	Age	Clinical diagnosis	Type of tachycardia*	Dosage of $MgSO_4$	Effect on tachycardia	Changes after injection
1	W. H.	72	Cor. scl.	p.a.t.	20 cc. of 20% sol.	+	Ventricular extrasystoles
					10 cc. of 10% sol.	−	
2	D. H.	50	Cor. scl.	p.v.t.	20 cc. of 10% sol.	+	
			Hypert., auric. fibril.	..	" " " " "	+	
3	V. J.	52	Gen. arteriosc.	p.a.t.	20 cc. of 20% sol.	+	Prolongation of P-R, ventricular extrasystoles
			Hypert., hemiplegia	..	" " " " "	+	Prolongation of P-R
					" " " " "	+	
					" " " " "	+	
4	J. G.	53	Pericard., Myocard.	a.f.	" " " " "	...	Alteration of flutter waves and a.v. conduction disturb., ventricular extrasystolea
5	H. S.	52	Luetic aortitis	p.a.t.	" " " " "	+	
6	M. W.	39	Rheum. mitral lesion	p.a.t.	20 cc. of 10% sol.	−	
					20 cc. of 20% sol.	+	Prolongation of P-R
					" " " " "	+	Prolongation of P-R
7	B. W.	42	Luetic aortic insuf.	p.a.t.	15 cc. of 10% sol.	−	
					20 cc. of 10% sol.	−	
					" " " " "	+	
8	V. K.	70	Rheum. mitral lesion	p.v.t.	" " " " "	−	
9	M. A.	60	Rheum. mitral lesion	p.a.t.	" " " " "	−	
10	D. S.	27	No organic ht. dis.	p.a.t.	" " " " "	−	
11	B. O.	51	No organic ht. dis.	p.a.t.	" " " " "	−	

* p.a.t. = paroxysmal auricular tachycardia; p.v.t. = paroxysmal ventricular tachycardia; a.f. = auricular flutter.

Observations. The results are summarized in Table 1. Twenty injections were given to 11 patients whose age varied between 27 and 72. Most of them had organic heart disease and some severe decompensation. Eight had paroxysmal auricular tachycardia, 2 paroxysmal ventricular tachycardia and 1 auricular flutter. Excluding the last, success was obtained in 6 of the 10 patients with paroxysmal tachycardia. In 1 instance each of 4 attacks was abolished by magnesium sulfate. Failures were limited to those individuals who received a 10% solution although this concentration sufficed for 1 patient with paroxysmal ventricular tachycardia and once in a case of paroxysmal

auricular tachycardia. Increases of volume of the solution seemed less important in obtaining good results than an increase of concentration.

All patients complained of a sensation of intense heat immediately following the injection and most of them perspired and became flushed; some complained of dizziness; nausea was frequent but vomiting was rare. General weakness was frequently noted for a short time after the injection.

In most cases the tachycardia ceased just before the injection was completed or immediately thereafter. Usually the attack stopped suddenly without any change in the electrocardiogram. This is evident in Figure 1*A* (Case 6). In this instance an auricular tachycardia with a rate of 186 disappeared during the injection of 20 cc. of a 20% solution of magnesium sulfate. If the effect is not immediate, none should be expected afterwards. In this respect the effect of magnesium sulfate resembles that of quinine or quinidine administered by the same route since the latter also acts at once or not at all. This also emphasizes the importance of the concentration.

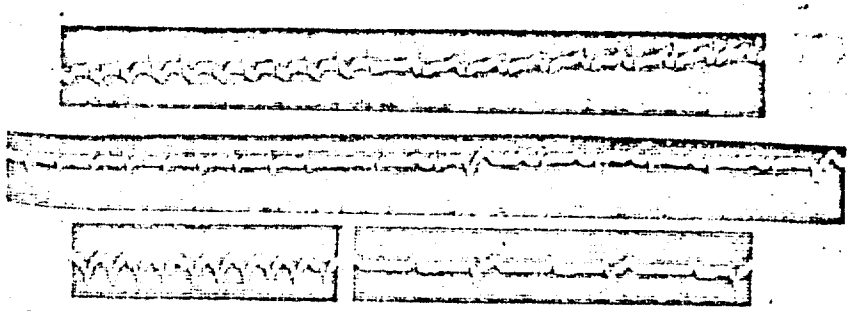

FIG. 1.—*A* shows the effect of an intravenous injection of magnesium sulfate in a case of paroxysmal auricular tachycardia; *B* shows a prolongation of the auriculoventricular conduction time and ventricular extrasystoles immediately after the injection of this agent in a case of paroxysmal auricular tachycardia; in *C*, a paroxysmal ventricular tachycardia disappears after the intravenous injection of magnesium sulfate.

In 3 patients the auriculoventricular conduction time of the sinus rhythm was lengthened immediately after the attack. The prolongation amounted to 0.02 to 0.05 second and persisted for a few seconds. This is exemplified by Figure 1*B* (Case 3). In this case of paroxysmal auricular tachycardia, each of 4 attacks were abolished by an injection of 20 cc. of a 20% solution of magnesium sulfate. The prolongation of the P–R interval however appeared only twice. A similar prolongation of the auriculoventricular conduction time was observed after 1 injection in Case 4 and 1 in Case 6.

Ordinarily the attack subsided abruptly without intermediate changes of rate or rhythm. In 3 cases, however, the rate of the tachycardia gradually slowed before it disappeared. In Case 3 it fell from 188 to 153 after each of 2 injections; in Case 5 it changed from 171 to 111 and in Case 6, 1 injection caused it to fall from 181 to 166 in 1 attack and from 193 to 176 in another.

Ventricular extrasystoles were noted after the injection (Fig. 1*B*) in 3 instances (Cases 1, 3 and 4). In 2 of them paroxysmal auricular tachycardia was present while auricular flutter was present in the other. In none of these cases were the ventricular extrasystoles of a type ever recorded in these patients previously.

The action of magnesium sulfate in a case of ventricular tachycardia is seen in Figure 1*C*. This 50 year old patient suffered from coronary sclerosis and auricular fibrillation. The first part of Figure 1*C* shows the ventricular tachycardia before, and the second part the fibrillation with single ventricular extrasystoles immediately after the injection of 20 cc. of a 10% solution of magnesium sulfate.

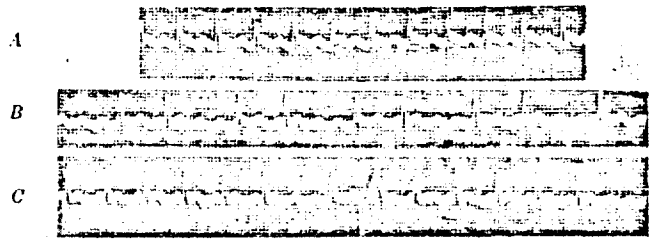

FIG. 2.—The tracings (Lead II), show the changes in the form of the F waves, ventricular extrasystoles and increased auriculoventricular block after the injection of magnesium sulfate in a case of auricular flutter.

The injection of 20 cc. of a 20% solution of magnesium sulfate did not produce any change in the flutter mechanism or the flutter rate in the single trial in this series. The shape of the F waves changed slightly presumably due to an intra-auricular block and the continuous 2:1 auriculoventricular block changed to a variable block with short periods of 4:1 block. This patient also had ventricular extrasystoles only when the magnesium preparation was given. The regular flutter with 2:1 block before the injection is evident in Figure 2*A*; Figure 2*B* and 2*C* show the changes in the F waves, the greater auriculoventricular block, and a ventricular extrasystole shortly after the injection. The electrocardiogram reverted to the appearance of Figure 2*A* within 4 minutes.

Discussion. The initial treatment of any attack of paroxysmal tachycardia should consist of a trial of the various vagal reflexes since these simple measures suffice to abolish the attack in a large percentage of the cases. Medicinal therapy is justified only when these reflexes (carotid sinus, bulbar, gag reflex, the Valsalva experiment) prove useless. Moreover the oral administration of quinidine is often successful although several hours or a few days may lapse before the result is obtained. It is not unusual, however, to encounter individuals who do not tolerate drugs of the quinine group although the attack must be abolished immediately owing to the development of severe anginal pain,[12] decompensation, or for some other reason. While the intravenous injection of quinine (quinidine) often yields an immediate and

satisfactory response, it is not always innocuous;[7] untoward symptoms have also appeared after the injection of mecholyl.[10] Therefore, the intravenous use of magnesium sulfate merits a definite place among these drugs in the medicinal management of these disorders.

The effect of bivalent electrolytes on cardiac automatism and stimulus formation varies markedly. Barium causes a very pronounced and stormy increase of stimulus formation; calcium acts similarly but is weaker and the action becomes evident only after much larger doses. Magnesium is antagonistic to calcium and inhibits stimulus formation; it disappears quickly from the blood but increased amounts cannot be recovered from the heart after an injection. Nevertheless a cumulative effect has been reported after injections are repeated at short intervals so that caution should be exerted in regard to dosage, if a second injection is given after a short interval.[2]

The appearance of extrasystoles under the influence of an inhibitory drug such as magnesium does not seem to have been previously observed in man but has been reported in the experimental animal.[4,9] The paradoxic occurrence of extrasystoles and even paroxysmal tachycardias after the administration of substances which ordinarily suppress them is a common observation; digitalis, potassium and quinine in moderate doses suppress extrasystoles while excessive amounts may be responsible for extrasystoles and even ventricular fibrillation.

The development of disturbed auriculoventricular conduction following administration of magnesium salts had to be anticipated on the basis on its depressing action and has been observed in animals.[11] In our patients it lasted only for a few seconds when the concentration of magnesium in the blood was high. While auriculoventricular conduction disturbances may be seen during or immediately after a tachycardia when the conduction system is fatigued, the observation of a similar disturbance during flutter, at the height of the magnesium effect, indicates that this must be attributed to the drug itself.

No untoward effects were encountered in our series. Some observers have employed a 30% solution without any accident.[14,15] Winkler, Smith and Hoff report a fatality after the injection of 30 cc. of a 25% solution without providing any details.[13] However, magnesium salts have often been given in concentrations of 10% to 30% by other workers for coronary sclerosis, angina pectoris and so forth without ill results. We would hesitate to employ the drug when marked myocardial damage is obvious, marked intraventricular conduction disturbances or gallop rhythm are present. Since the concentration in which the drug reaches the heart is important for the accomplishment of the effect, the injection should not be given too slowly; theoretically injection of higher concentrations might damage the heart if it is given too rapidly so that a moderately rapid injection is suggested. About 30 seconds should be taken for the injection. The employment of 15 to 20 cc. of a 20% solution promises greater success than larger volumes of a 10% solution. No important change of sinus rhythm followed the amounts given by us. The difference of response of a normal automatic center and an abnormal extrasystolic center is a

matter of common observation and the difference in doses necessary for dogs and man has been previously emphasized.[6]

While we can confirm the observation that digitalis extrasystoles are abolished by magnesium sulfate, the effect lasts only for a few minutes.[15] Perhaps some paroxysmal tachycardias of multifocal origin which appear after large doses of digitalis or injections of strophanthin and which, unfortunately, sometimes change into ventricular fibrillation may be influenced by injections of magnesium sulfate whereby a dangerous accident might be averted.

Conclusions. The effect of intravenous injections of magnesium sulfate in 10 cases of paroxysmal tachycardia and 1 case of flutter was studied.

The injection of a 10% solution was beneficial in 3 out of 8 attacks, while a 20% solution succeeded in 8 out of 8 attacks. Consequently the use of a 20% solution is advocated.

Disturbances of conduction and ventricular extrasystoles appear for a short time after the injection. The rate of the paroxysmal tachycardia frequently diminishes before the tachycardia disappears.

In the doses and with the indications discussed, the intravenous injection of magnesium sulfate may be recommended as a useful therapeutic procedure in paroxysmal tachycardias.

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Spectrophotometric Determination of Magnesium in Blood Serum Using Magon

Patricia J. Burcar, Albert J. Boyle, and Robert E. Mosher

A spectrophotometric method for the determination of serum magnesium using Magon, 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)naphthalene-1′-(2-hydroxybenzene), is presented. The serum is prepared by a nitric-perchloric acid digestion. Interfering phosphate is removed as aluminum phosphate. The colored complex is developed at pH 11.2 in an alcoholic medium and read at 505 mμ.

PRESENT METHODS for the determination of magnesium in biologic fluids and their inadequacies have been reviewed by MacIntyre and Wootton (*1*). In 1957, Mann and Yoe (*2*) introduced a color reagent for magnesium called Magon, 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)naphthalene-1′-(2-hydroxybenzene), which they reported has a sensitivity 8 times greater than that of Titan yellow. Since then, Magon has been used for magnesium determinations in sea water, brine (*3*), and rocks (*4*). The extreme sensitivity of this azo dye encouraged us to investigate further the properties of Magon and its application to the determination of serum magnesium.

Present Study

Apparatus

Spectrophotometer For this study a Coleman Universal spectrophotometer with round cuvets, 19 × 105 mm. was used.

Digestion tubes Universal tubes, 200 × 25 mm., Corning No. 7920, graduated at 12.5, 25, 35, and 50 ml., or Folin-Wu receiving tubes of the same dimensions calibrated at only 12.5 and 25 ml., Corning No.

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7900, were used for both digestion and color development. It is desirable to recheck the 12.5-ml. calibration on these tubes and place them in groups with 2% tolerance.

Reagents

Magon reagent Place 100 mg. of Magon in a 1-L. volumetric flask. Add approximately 500 ml. of 95% ethanol and warm gently with stirring. Cool and dilute to 1 L. with 95% ethanol. Filter through Whatman No. 1 filter paper to remove undissolved particles. (Magon can be obtained from LaMotte Chemical Products Co., Chestertown, Md.) If denatured alcohol is used, an individual check must be made to determine whether or not the denaturant present will interfere.

Sodium hydroxide Prepare a solution containing 2 gm. of reagent-grade NaOH in 100 ml. of deionized water.

Succinate buffer Place 2 gm. of reagent-grade succinic acid into a 150-ml. beaker and dissolve with 75 ml. of deionized water. Adjust the pH to 5.5 with NaOH. Transfer to a 100-ml. volumetric flask and dilute to volume with deionized water.

Borate buffer Dissolve 3 gm. of reagent-grade sodium borate ($Na_2B_4O_7 \cdot 10H_2O$) in 75 ml. of deionized water. Adjust the pH to 9.5 with NaOH. Transfer the solution to a 100-ml. volumetric flask and dilute to volume with deionized water.

Methyl red Dissolve 10 mg. in 100 ml. of 95% ethanol.

Aluminum chloride solution Dissolve 8 gm. of reagent-grade aluminum chloride hexahydrate in 100 ml. of deionized water.

Magnesium stock solution Add 200 mg. of distilled magnesium metal to a 100-ml. volumetric flask. Dissolve, using the minimal quantity of hydrochloric acid. Dilute to volume with deionized water.

Magnesium working standard Transfer 3 ml. of the stock solution to a 500-ml. volumetric flask and dilute to volume with deionized water. This gives a concentration of 12 μg./ml.

Standard calcium solution Transfer 250 mg. of reagent-grade calcium carbonate to a 1-L. volumetric flask and dissolve in a minimum amount of hydrochloric acid. Dilute to volume with deionized water. This solution contains 100 μg. of calcium per milliliter.

Preparation of Sample

Pipet 1 ml. of serum into a digestion tube. Add 2 ml. of concentrated nitric acid and 2 drops of concentrated perchloric acid. Place the tube in an aluminum heating block (5) at 130° to 150°. The initial tempera-

ture of the aluminum block is 120°. When the volume is reduced to about 1 ml., turn the hot plate to high (180–210°). Continue the digestion until the perchloric acid fumes. Add 3 drops of nitric acid and leave on the block until dry salts start to form.

Remove from the block and wash down the walls of the tube with approximately 2–4 ml. of deionized water. Add 1 ml. of succinate buffer, 2 drops of methyl red, and 1 drop of aluminum chloride. Add sodium hydroxide dropwise from a polyethylene dropping bottle until the methyl red just turns yellow. Place the tubes in a boiling water bath for 5 min. Remove, cool, and dilute to 12.5 ml. with deionized water. Centrifuge and transfer two 2-ml. aliquots of the supernatant liquid to separate calibrated tubes. Add 5 ml. of Magon solution and mix. Add 0.5 ml. of borate buffer, mix, and dilute to 12.5 ml. with 95% ethanol. Transfer to a cuvet and read at 505 mμ against a reagent blank.

Preparation of Standards

Prepare standards to contain 6.0 to 42 μg. of magnesium using the working standard and add 1 ml. of the standard calcium solution to each. Prepare the blank without calcium. Treat both standards and blank in the same manner as samples and prepare a standard calibration curve.

Results and Discussion

Optimum Conditions

The basic properties of Magon have been described by the work of Mann and Yoe (*2*). The most suitable wavelength was found to be 505 mμ, where there is a maximum differential between the absorption of the magnesium complex and Magon. At 620 mμ, only Magon absorbs. The determination of the unreacted Magon, using absorption measurements at this wavelength, was investigated to determine whether this would serve as a means of measuring magnesium, but this approach was abandoned because interfering substances tended to enhance error at this wavelength. At 505 mμ, the different coefficients of absorption of Magon and complex result in a standard curve which is not strictly linear unless correction is made for the unreacted dye. The deviation from linearity is minor and for practical purposes the uncorrected curve was used. Since unknowns are measured in the same way as the knowns with which they are compared, there can be no error introduced due to unreacted reagent. There is, however, a loss in sensitivity to some degree. The amount of dye needed for the completion of the reaction of 11 μg. of magnesium was 600 μg. of Magon or about a

1:3 molar ratio. The complex formation is stable with respect to time and through a temperature range from 25° to 55°. At room temperature the magnesium complex remains constant for 3 days; Magon solutions are stable for at least 1 month. These observations agree with those of Mann and Yoe (*2*).

The optimum conditions for complex formation depend on close control of pH and ethanol content. The spectra of both Magon and the Magon-magnesium complex are dependent on the pH and the ethanol content. The ethanol is needed to keep the Magon in solution. Furthermore, the pH of a given buffer system is dependent on the ethanol content. Maximum magnesium complex development is desired with

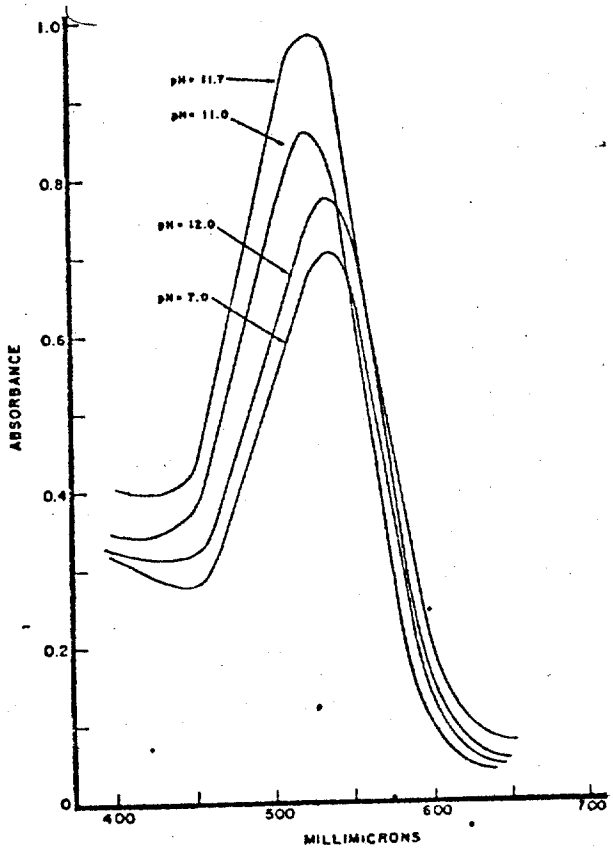

Fig. 1. Effect of pH on magnesium-Magon spectra. Variation with pH of 1000 μg. of magnesium and 500 μg. of Magon in 25 ml. of 70% ethanol.

minimal dye absorbance and interaction with other materials. As shown in Fig. 1, the maximum development of the magnesium complex lies between pH 11 and 12. On the other hand, the minimum color from unreacted dye lies in the range from pH 10 to 11 (Fig. 2).

Figure 3 illustrates graphically the strong interdependence of the complex, pH, and ethanol content. The relationship seems to be of a ternary nature in that ethanol content can influence the complex, pH can influence the complex, and ethanol content can influence the pH.

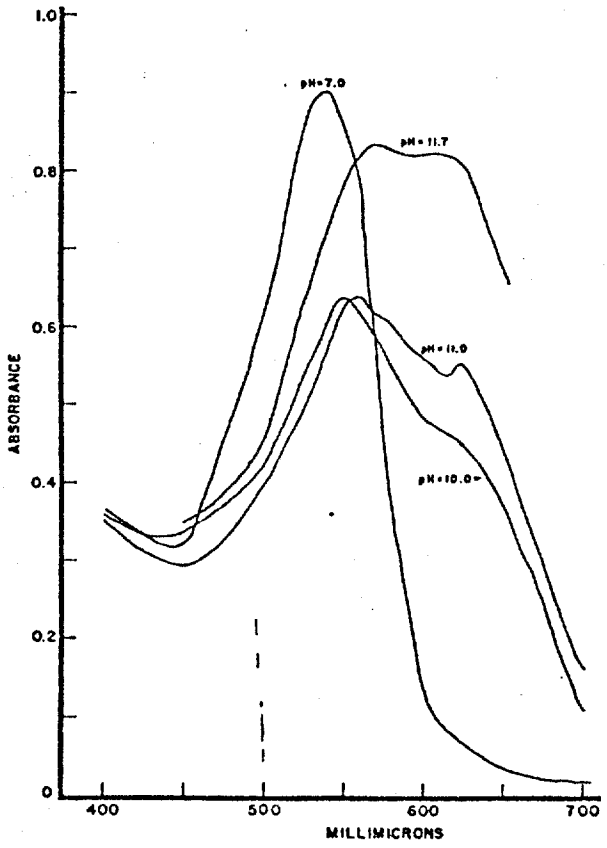

Fig. 2. Effect of pH on Magon spectrum. Variation with pH of 500 μg. of Magon in 25 ml. of 70% ethanol.

Calcium interference is also dependent on both water and pH. With increasing pH and/or water content, the interaction between Magon and calcium increases significantly. This is emphasized in Fig. 4. To minimize the calcium interference and retain good magnesium sensitivity, a pH of 11.1–11.3 and an ethanol content of 76% were used. Adherence to these optimal conditions is necessary for reproducible results.

Digestion

Protein must be removed to avoid precipitation in the alcoholic medium during color development. Wet-ashing seemed to be the most

feasible approach. It was found that 1 ml. of serum could be completely digested with minimum amounts of nitric and perchloric acids provided combustion tubes were used. In order to remove ammonium salts which form during the breakdown of protein, a few additional

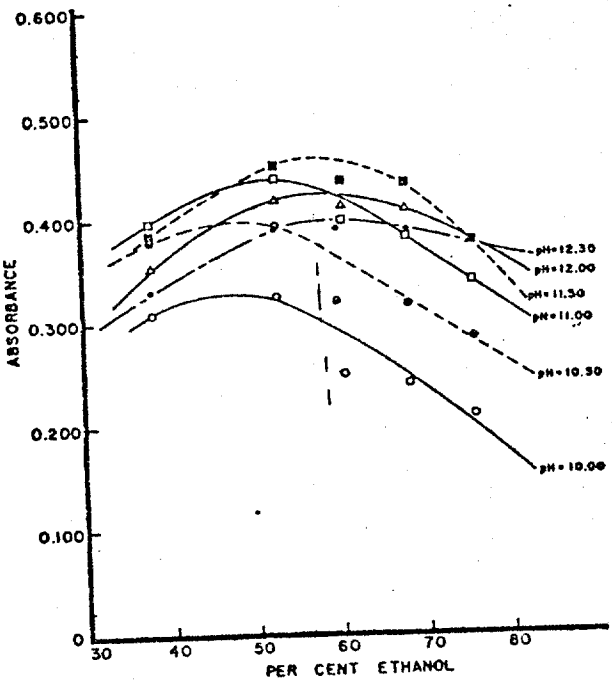

Fig. 3. Variation with pH and ethanol content of 4.18 μg. of magnesium and 500 μg. of Magon in total volume of 12.5 ml.

drops of nitric acid are added to the sample toward the end of the digestion. All chlorides are volatilized during this digestion. No care need be taken to remove oxidizing agents after wet ashing, since hydrogen peroxide was found to cause no change in the complex. If the hot plate and aluminum block are preheated to 120°, the digestion takes about 3 hr.

Interferences

The interference of ions present in serum in concentrations greater than 0.5 μg./ml. was considered. These include Fe (0.1–1.8 μg./ml.), Cu (1.96–2.63 μg./ml.), Ca (90–110 μg./ml.), NH_4 (1.43–3.0 μg./ml.), P (85–155 μg./ml.), S (22–56 μg./ml.), Cl (3500–3710 μg./ml.) (*6*), Zn (1.25 μg./ml.) (*7*). The sensitivity of Magon makes it possible to use an aliquot equivalent to 0.16 ml. of serum and the quantities of ions checked were based on these relationships.

Serum phosphate was found to cause the major interference, as

shown in Table 1. In an effort to understand the nature of this interference, certain factors were investigated. This effect cannot be due exclusively to a magnesium phosphate complex since Magon is affected by phosphate even in the absence of magnesium, as shown in Fig. 5.

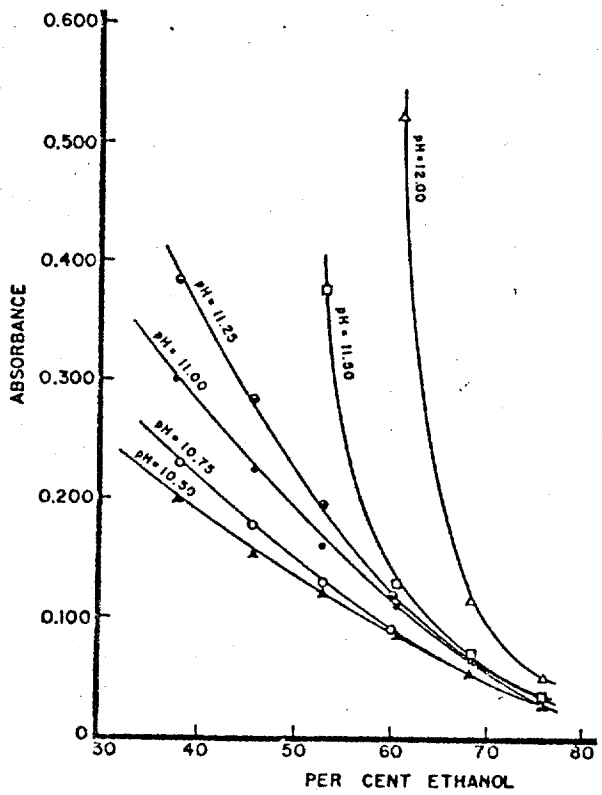

Fig. 4. Variation with pH and ethanol content of 43 μg. of calcium and 500 μg. of Magon in total volume of 12.5 ml.

Phosphate does not produce a pH change and the interference is not dependent on ionic strength. Environmental changes such as order of addition and higher pH will reduce but not eliminate the interference. Its nature is unknown.

Investigations to mask or remove the phosphate led to the discovery that aluminum effectively precipitates all the phosphate at a pH of 5.5–6.5 (Table 2). This was confirmed by spectrographic analysis. The excess precipitant comes down as the hydroxide. Iron is also removed. At least 1 mole of aluminum is required per mole of phosphorus but large excesses must be avoided. Boiling is necessary for complete precipitation and reduces the inclusion of magnesium in the precipitate. Although aluminum has been reported to interfere (*2*), this controlled precipitation is complete enough to avoid such problems.

Table 1. Interferences, Ions Present in Serum

Ion		Magnesium	
		Present	Found
		μg. per 12.5 ml.	
Cu	0.05	4.18	4.18
Zn	0.05	4.18	4.18
Ca	20.0	6.27	5.70
	20.0	4.18	3.70
	20.0	2.09	1.95
	20.0	2.09	2.45
	20.0	3.14	3.52
	20.0	4.18	4.40
	20.0	5.23	5.35
	21.5	4.18	4.08
	40.0	4.18	3.83
NH_4	30.0	8.84	8.75
	60.0	8.84	8.50
	180.0	8.84	8.00
P (as PO_4)	3.2	5.65	4.45
	6.4	5.65	4.77
	12.8	5.65	4.55
	19.2	5.65	4.72
	25.6	5.65	4.35
	25.6	5.65	5.02
	51.2	5.65	5.02
	12.8	2.83	1.70
	35.6	2.83	2.65
S (as SO_4)	11.0	4.18	4.23
ClO_4	1.180 gm.	3.54	3.65
Cl	3.55 mg.	8.84	9.00

A succinate buffer with methyl red as the indicator is used to maintain pH 5.5–6.5 for the aluminum precipitation. It is impossible consistently to add sodium hydroxide alone to the strongly acid solution obtained from the digestion and stay within the narrow pH range needed for complete aluminum precipitation. A buffer is necessary to insure the correct pH and avoid re-solution of the aluminum which occurs at pH values greater than 6.5. Other buffers were tried with limited success but succinate has several distinct advantages. The precipitate formed centrifuges well. A small quantity of succinate is sufficient to maintain the pH for aluminum precipitation and yet permits the pH of the solution to be raised later for the final color development by a second, more concentrated, buffer.

Table 2. PHOSPHATE REMOVAL

Phosphorus (*μg. per 12.5 ml.*)	*Magnesium* (*μg. per 12.5 ml.*)	
	Present	*Found*
0	7.30	7.27
155	7.30	7.20
0	6.40	6.40
155	6.40	6.35

Note: Aluminum and succinate were present in all samples.

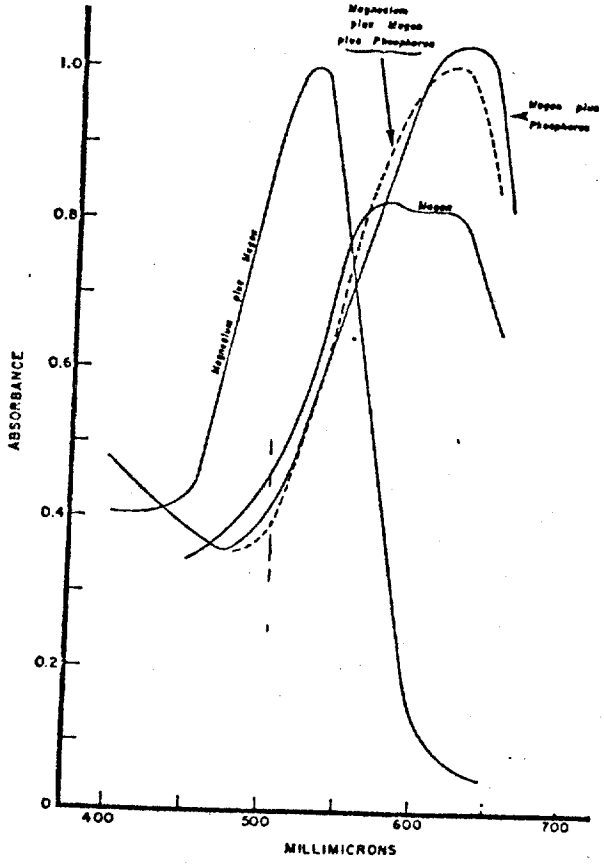

Fig. 5. Effect of phosphorus. From left to right: 1000 μg. magnesium and 500 μg. of Magon; 500 μg. of Magon: 8.84 μg. of magnesium, 155 μg. of phosphorus, 500 μg. of Magon; 500 μg. of Magon, 155 μg. of phosphorus. All spectra obtained at pH 11.75 and with an ethanol content of 85.5%.

The magnitude of the calcium interference, which was a factor in selecting optimum conditions, is illustrated in Table 1. The interference, which appears to be inconsistent, is compensated for by adding calcium to a standard curve developed at the time of analysis. Table 3 represents values of such a curve obtained on two different days.

Table 3. Stability of Standard Curve

Magnesium (μg. per 12.5 ml.)	Absorbance at 505 mμ	
	4/22/63	5/7/63
2.01	0.195	0.199
3.02	0.289	0.319
4.02	0.361	0.367
5.03	0.436	0.449
6.03	0.519	—

Table 4. Recoveries on Human Serum

Magnesium (mg. per 100 ml. serum)			
Serum base	Added	Anticipated	Found
1.59	1.25	2.84	3.05
1.59	1.25	2.84	3.05
1.59	1.25	2.84	2.99
1.59	0.627	2.22	2.31
1.59	0.627	2.22	2.16
1.59	0.627	2.22	2.38
0.795	2.51	3.31	3.36
0.795	2.51	3.31	3.14

Average recovery: 103.0 ± 3.88%.

Ammonium ion in concentrations higher than those found in serum does cause an interference and this precludes the use of ammonium hydroxide for neutralization.

Precision and Recoveries

The average deviation of 17 determinations on a single serum sample was ±3.94% with 8 of these determinations having a variability under 2%. Recovery studies were made by adding known quantities of magnesium to a serum (Table 4).

Serums were analyzed from 21 apparently normal subjects, aged 19–69, with the majority between 20 and 45 years. The range was 1.51–2.28 mg./100 ml. with an average value of 2.00 mg./100 ml.

Summary

A method for serum magnesium has been presented using Magon. The sensitivity of the reaction is such that: (1) the measurement of 3.5 μg. magnesium with an absorbance of about 0.250 is possible and (2) multiple aliquots may be developed on only 1 ml. of serum if de-

sired. A digestion step is necessary but if done on an aluminum block does not require continual attention. The average value obtained on analysis of 21 normal subjects is 2.00 mg./100 ml. with a range from 1.51 to 2.28 mg./100 ml.

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SOME EFFECTS OF MAGNESIUM LOADING UPON RENAL EXCRETION OF MAGNESIUM AND CERTAIN OTHER ELECTROLYTES[1]

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Mendel and Benedict (1) injected magnesium salts into animals and found that nearly all of the magnesium could be recovered in the urine. Incidentally, they noted that magnesium injections were followed by "a noteworthy rise in the urinary output of calcium." Schwartz, Smith and Winkler (2) made some measurements of the magnesium clearance in their study of the renal excretion of sulfate by dogs. They found that the renal clearance of magnesium was always less than that of sulfate and, therefore, "presumably the excretion of a small amount of some other cation was stimulated, in order to ensure continuous electroneutrality of urine." Chloride excretion was "almost completely repressed by sulfate excretion." In control periods, in three dogs, the renal clearances of magnesium were 2, 1 and 2 ml. per minute. Following the injection of magnesium sulfate, in five dogs, the magnesium clearances ranged from 12 to 24 ml. per minute with magnesium to creatinine clearance ratios ranging from 0.293 to 0.45.

Several investigators (3–6) have since found that the magnesium clearance increases after the injection of magnesium salts, although what appears to be the only study systematically relating clearances to plasma concentrations of magnesium has been published only in abstract form (7, 8).

Magnesium clearances, which usually have been calculated as $U_{Mg}V/P_{Mg}$, are not a simple reflection of how the kidney handles this element. Copeland and Sunderman (9), as well as others whose work they review, have shown that a third or more of the plasma magnesium is bound to proteins and therefore not filtrable at the glomerulus. Willis and Sunderman (10) have published nomograms from which filtrable magnesium and magnesium concentrations in ultrafiltrates of serum may be derived from the total magnesium and protein concentrations in serum.

The present paper extends the observations reported by others by studying the effects of magnesium over a wider range of serum concentrations, over rising, constant and falling serum levels, and in subjects whose glomerular filtration rates ranged from 25 to 196 ml. per minute.

MATERIAL AND METHODS

Thirty-four women served as subjects of the study. None of the five nonpregnant women had any discernible disturbance in hydration or cardiovascular-renal function. The remaining 29 women were selected with a view to getting cases with a wide range of inulin clearances, with representatives throughout the range. These women were all pregnant and were distributed as follows: "normal", 4; preeclampsia, 14; hypertensive disease, 7; renal disease, thought to be chronic glomerulonephritis, 4. The patients with renal disease all had hypertension, proteinuria and renal impairment. The one who did not have edema, and who had not been seen here in a previous pregnancy, was in uremia and had a urea clearance of 3 ml. per minute. Another had a urea clearance of 30 ml. per minute and the remaining two had inulin clearances of 25 and 52 ml. per minute, respectively.

All patients were on an 1,800 calorie diet, which contained about 30 mEq. of sodium and 90 mEq. of potassium. However, some had been in the hospital for only a day or two while others had been in for as long as 29 days before the tests were done.

Two salts of magnesium were used, the sulfate and the acetate.

Procedures (a). In the six experiments with magnesium acetate, the fasting subjects were hydrated with oral water in the early morning. At about 9 a.m. a multiple-eyed catheter was placed in the bladder and an infusion of dilute inulin and sodium para-aminohippurate (PAH) in 0.9 per cent saline was started. The concentrations of inulin and PAH depended upon the anticipated levels of the clearances of these substances. Priming doses of 3 Gm. of inulin in 10 per cent solution and 0.2 Gm. of PAH in 20 per cent solution were injected through the infusion tubing. The infusion was given at the rate of 3.7 ml. per minute, using a Bow-

[1] Supported by Research Grant H-1837 from the National Institutes of Health, Public Health Service.

man pump. After allowing 45 minutes for equilibration, three control clearance periods were run for inulin, PAH and magnesium and for the urinary excretion rates of sodium, potassium, calcium and chloride. As soon as the last control urine had been taken, 100 ml. of 10 per cent magnesium acetate [93.2 mEq. of magnesium in $Mg(C_2H_3O_2)_2 \cdot 4\ H_2O$] were added to the 350 ml. (*circa*) of remaining infusion solution and the blood and urine collections were continued at intervals of 15 to 30 minutes until all of the infusion solution had been given. The rate of magnesium infusion was about 0.8 mEq. per minute.

Venous blood samples were taken midway between urine collections and allowed to clot. At each urine collection, the bladder was washed out with 20 ml. of distilled water and blown out with 50 cc. of air. The patients lay on their backs, in low Fowler's position, throughout the tests.

In the experiments with magnesium sulfate, several variations were tried.

(*b*). After three control periods as above, 2 Gm. of magnesium sulfate (16.2 mEq. of magnesium in 10 per cent $MgSO_4 \cdot 7\ H_2O$) was injected intravenously and enough added to the infusion solution to give about 20 to 30 mEq. of magnesium per liter and an infusion rate of 70 to 100 μEq. of magnesium per minute. After allowing 30 minutes for equilibration, three more clearance periods were run. Then another 2 Gm. of magnesium sulfate was given intravenously and the sustaining solution brought to about 41 mEq. per liter, giving an infusion rate of 0.15 mEq. of magnesium per minute. Again, 30 minutes was allowed for equilibration and then three more clearance periods were run. Three patients were studied in this way, each at three plateaus of serum magnesium concentration. The total dose of magnesium sulfate, as $MgSO_4 \cdot 7\ H_2O$, amounted to about 6.5 Gm. over a period of about three hours.

(*c*). After three control periods as above, 10 Gm. of magnesium sulfate (81.2 mEq. of magnesium) in 50 per cent solution was injected intramuscularly and the observations continued for at least four hours. In this variation the serum magnesium concentrations rose for 90 to 120 minutes, were nearly constant for the next 90 to 120 minutes, then fell off. Seven patients had concurrent measurements of inulin and PAH clearances and 12 others did not.

(*d*). In six patients the serum concentrations of magnesium were raised markedly and acutely and then held nearly constant for three to four hours by injecting almost simultaneously 3 Gm. of magnesium sulfate intravenously and 10 Gm. intramuscularly (total of 105.5 mEq. magnesium). Inulin and PAH clearances were not done in this group.

Complete protocols for representative experiments are shown in Table I.

Magnesium was measured in duplicate in serum and urine by the method of Simonsen, Westover and Wertman (11); it was often necessary to dilute the urine samples, by as much as twentyfold after large doses of magnesium. Inulin was measured in duplicate or quadruplicate by the method of Roe, Epstein and Goldstein (12). Urinary chlorides were titrated by the method of Volhard and Harvey (13). Sodium and potassium were measured with a Perkin-Elmer 52C flame photometer, using an internal lithium standard. Calcium was measured in duplicate by the method of Tisdall and Kramer (13).

The magnesium clearances, as presented, are approximations because the plasma levels of magnesium were changing during many of the observations. The expression "serum magnesium" will be used henceforth, for this is what was measured. In some contexts, this expression will be equated with plasma magnesium. "Corrected" magnesium clearances are those calculated from the filtrable magnesium, as derived from the nomogram of Willis and Sunderman (10). Whether or not their nomograms can be used legitimately will be taken up in the Discussion.

RESULTS

Effect of parenteral magnesium upon urine flow and clearances of inulin and PAH

As may be seen in the last two columns of Table II, there was no consistent effect of magnesium upon the rate of urine flow or upon the clearances of inulin and PAH. The fluid intake was constant at 3.7 ml. per minute, by infusion. No comparison of the acetate and sulfate salts was attempted, because there are too few cases. The first three of the four procedures described in the Methods section are represented in the table but were not analyzed separately, again because the cases are so few. Data are shown only for the first four (sometimes three) clearance periods in order to average the same number of observations for each period.

Considering all cases together, the average changes were small. This has been the finding of previous investigators. For instance, Heller, Hammarsten and Stutzman (3), who gave magnesium sulfate to six normal young men, found an average decrease of 9.9 per cent in the PAH clearance and an average decrease of 8 per cent in the inulin clearance. Womersley (4) found no effect of magnesium sulfate upon the inulin clearance in five normal men. Etteldorf, Clayton, Tuttle and Houck (14) loaded 15 normal children with magnesium sulfate and found no significant changes in either PAH or inulin clearances. Pritchard (15) found no significant effect upon these clearances in seven women with preeclamp-

TABLE I

Representative experiments on renal effects of magnesium loading

Subject	Time	Urine flow	Inulin clearance	PAH clearance	Serum Mg	Excretions (μEq./min.)					Mg clearance
						Na	Cl	K	Ca	Mg	
	min.	*ml./min.*	*ml./min.*	*ml./min.*	*mEq./L.*						*ml./min.*
1*	0	Prime: Inulin, 3 Gm. and PAH, 0.4 Gm.									
	Mean of 3 controls	Sustaining infusion A: Inulin, 88 mg./min. and PAH, 31.5 mg./min. in 0.9% NaCl solution at 3.7 ml./min.									
	45–102	9.89	141	684	1.58	327	367	105	3.3	9.0	5.7
	103	Sustaining infusion: 93.2 mEq. Mg in 10% $Mg(C_2H_3O_2)_2 \cdot 4\ H_2O$ solution added to A									
	102–123	10.29	153	727	1.79	322	364	106	3.6	15.2	8.5
	123–144	12.57	174	724	2.64	404	490	89	12.9	84.3	31.9
	144–162	11.33	169	706	2.92	468	481	77	17.5	113.0	38.7
	162–184	10.63	173	750	3.64	534	546	80	21.6	180.0	49.5
	184–204	13.20	184	744	4.03	454	722	89	30.1	237.0	58.8
2†	0	Prime: Inulin, 3 Gm. and PAH, 0.4 Gm.									
	Mean of 3 controls	Sustaining infusion A: Inulin, 53 mg./min. and PAH, 21 mg./min. in 0.9% NaCl solution at 3.7 ml./min.									
	45–88	5.66	60	421	1.83	234	164	60	3.3	3.3	1.8
	90	Prime: 16.2 mEq. Mg in 10% $MgSO_4 \cdot 7\ H_2O$ solution Sustaining infusion B: 5.7 mEq. Mg in 10% $MgSO_4 \cdot 7\ H_2O$ solution added to A									
	122–135	5.08	55	414	3.12	237	139	33	11.2	27.1	8.7
	135–150	4.80	48	345	3.26	260	140	29	12.4	31.6	9.7
	150–167	4.70	43	306	3.38	254	161	30	14.0	30.8	9.1
	170	Prime: 16.2 mEq. Mg in 10% $MgSO_4 \cdot 7\ H_2O$ solution Sustaining infusion C: 16.2 mEq. Mg in 10% $MgSO_4 \cdot 7\ H_2O$ solution added to 400 ml. of A									
	200–211	5.45	47	311	4.82	327	233	30	27.7	78.6	16.3
	211–224	4.47	44	286	4.50	289	192	26	24.1	64.8	14.4
	224–238	4.29	46	292	4.64	302	205	29	24.7	62.2	13.4
3‡	0	Prime: Inulin, 3 Gm.									
	Mean of 3 controls	Sustaining infusion: Inulin, 88 mg./min. in 0.9% NaCl solution at 3.7 ml./min.									
	48–104	5.06	121		1.68	47	48	16	3.8	5.7	3.4
		81.2 mEq. Mg in 50% $MgSO_4 \cdot 7\ H_2O$ solution, intramuscularly									
	104–127	4.17	107		2.02§	79§	64§	14	12.7§	19.6	9.7§
	127–148	4.00	115		2.71	97	110	19	16.4	46.3	17.1
	148–172	3.08	99		3.26	99	111	18	36.4	139.5	42.8
	172–194	3.37	103		3.75	108	115	19	42.1	172.0	45.9
	195	40.6 mEq. Mg in 50% $MgSO_4 \cdot 7\ H_2O$ solution, intramuscularly									
	194–222	2.43	96		4.47	133	102	19	46.6	215.5	48.2
	222–244	2.41	107		5.00	152	136	21	64.4	317.5	63.5
4‖	Mean of 3 controls										
	0–118	1.92			1.69	74	44	13	1.1	6.1	3.6
	118–125	24.3 mEq. Mg in 10% $MgSO_4 \cdot 7\ H_2O$ solution intravenously, plus 81.2 mEq. Mg in 50% $MgSO_4 \cdot 7\ H_2O$ solution intramuscularly									
	137–192	2.00			3.75	268	154	17	10.6	202.0	53.9
	192–252	1.75			4.16	183	143	11	11.9	177.0	42.5
	252–312	2.20			4.01	152	129	14	15.8	252.0	62.8
	312–376	1.00			3.53	73	76	5	10.5	196.5	55.7
	377–380	40.6 mEq. Mg in 50% solution of $MgSO_4 \cdot 7\ H_2O$ solution intramuscularly									
	376–437	3.43			3.44	36	51	9	7.4	159.0	46.1

* Case of essential hypertension in the thirty-ninth week of gestation. Procedure (*a*), described in section on methods.

† Case of essential hypertension in the thirty-second week of gestation. Procedure (*b*), described in section on methods.

‡ Nonpregnant "normal" patient. Procedure (*c*).

§ Not used because of uncertainty as to mean serum magnesium concentration in this period.

‖ Case of severe preeclampsia in the thirty-sixth week of gestation. Procedure (*d*).

TABLE II

Rates of urine flow (V), inulin clearance (C_I) and para-aminohippurate clearance (C_{PAH}) during and after injection of magnesium salts

Group	Cases	Control	Periods during or after Mg injection				Periods with	
			1	2	3	4	Increase†	Decrease†
Normal		*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*	%	%
V	5*	4.84	4.06	3.97	3.94	4.41	25.0	75.0
C_I	5*	127	108	107	110	118	5.0	40.0
C_{PAH}	1*	689	770	602	796		66.7	33.3
Preeclampsia								
V	4	3.85	3.85	3.14	3.77	3.82	37.5	56.3
C_I	4	98	103	124	115	110	31.2	37.5
C_{PAH}	4	422	418	351	417		50.0	8.3
Hypertension								
V	5	5.32	5.05	5.73	6.33	5.62	25.0	20.0
C_I	5	89	86	92	90	86	15.0	25.0
C_{PAH}	5	357	373	552	485	517	10.0	15.0
Renal Disease								
V	2	1.21	1.34	1.31	1.43	2.33	62.5	25.0
C_I	2	40	56	51	53		50.0	50.0
C_{PAH}	2	260	348	281	280		33.3	33.3
Totals								
V	16	4.29	3.97	3.98	4.33	4.39	32.8	46.9
C_I	16	98	94	100	98		19.4	35.4
C_{PAH}	12	390	417	444	454		29.3	17.1

* One normal pregnancy.
† Changes greater than 10 per cent.

sia, who were given 4 to 6 Gm. of magnesium sulfate intravenously.

Effect of magnesium upon the uncorrected magnesium clearance

The relationship between the total serum magnesium and the uncorrected renal clearance of magnesium is shown in Figure 1. The eight patients represented here were selected as "normals" in that they gave no evidence of disturbance in hydration or cardiovascular or renal function. There was a roughly linear increase in the magnesium clearance with rising serum concentrations of magnesium, both when magnesium sulfate and when magnesium acetate were given.

Figure 2 shows the same relationship in patients selected for a wide range in the inulin clearance. One woman in the terminal stage of glomerulonephritis and azotemia, with a urea clearance of 3 ml. per minute (inulin clearance not measured), had only a slight increase in the magnesium clearance (from 1.2 to 2.8 ml. per minute) when the serum concentration was increased from 2.1 to 4.4 mEq. per liter. At each level of glomerular filtration, the magnesium clearance increased as a linear function of the serum magnesium concentration (roughly, as in Figure 1).

Ratio of "corrected" magnesium clearance to inulin clearance

The data shown in Figure 3 are from 16 patients in whom inulin and magnesium clearances were measured simultaneously. These patients were selected to cover a wide range of inulin clearances, which, in the control periods, varied from 25 to 196 ml. per minute. The "corrected" magnesium clearances, therefore, varied widely. However, calculation of the ratio of the corrected magnesium clearance to the inulin clearance brought these data to a common base and Figure 3 indicates that the effects of the two magnesium salts were about the same, that is, the serum magnesium concentration rather than the attendant anion seems to determine the renal excretion of magnesium. It

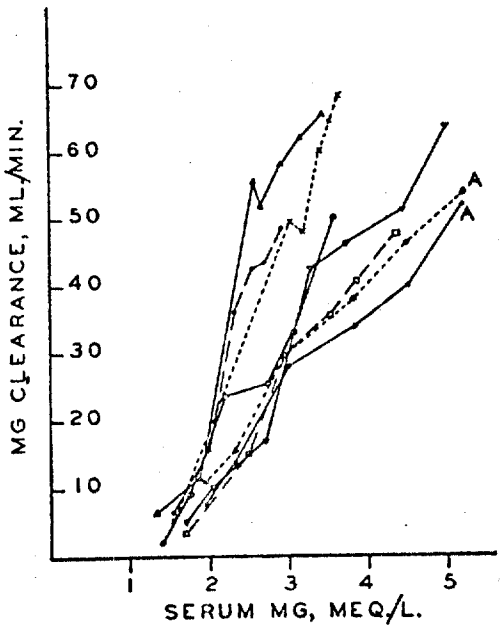

FIG. 1. THE RELATIONSHIP BETWEEN THE MAGNESIUM CLEARANCE, UNCORRECTED FOR PROTEIN BINDING OF SERUM MAGNESIUM, AND THE SERUM CONCENTRATION OF TOTAL MAGNESIUM

Eight "normal" subjects. A denotes subjects given magnesium acetate [Procedure (*a*)]; the others were given magnesium sulfate [Procedure (*c*)].

also shows that the moderately impaired kidney handled magnesium as did the normal one—that is, the tubular reabsorption or rejection of magnesium per 100 ml. of glomerular filtrate was similar from patient to patient. The triangles in Figure 3 denote observations in patients with glomerulonephritis and inulin clearances of less than 60 ml. per minute.

At the serum concentrations of magnesium studied, the clearance ratio of "filtrable" magnesium to inulin rose steeply as the serum concentration of magnesium increased. The clearance ratio approached but did not reach 1. This suggested the possibility that tubular reabsorption of magnesium might have been nearly maximal under "basal" conditions and that increments in the filtered load were excreted almost completely. Figure 4 indicates that this may have been so in 13 of the 16 patients studied. The filtered load was calculated by multiplying the inulin clearance by the concentration of magnesium in ultrafiltrate, as derived from the appropriate nomogram of Willis and Sunderman (10). In Figure 4, the increases in magnesium excretion over control values do seem to bear a 1 to 1 ratio to the increments in the calculated filtered loads, for most of the points fall close to the line describing such a relationship. Three abnormal patients (one with essential hypertension and two with preeclampsia) deviated widely from the general pattern, in that all observations fell far below the line. These patients are indicated in the graph by the broken lines connecting their points.

Tubular reabsorption of magnesium

If the nomogram of Willis and Sunderman (10) can be used legitimately for the derivation of magnesium concentrations in ultrafiltrates of plasma, Figure 4 describes the tubular reabsorption of magnesium. Points falling above the line would represent decreases in tubular reabsorption under

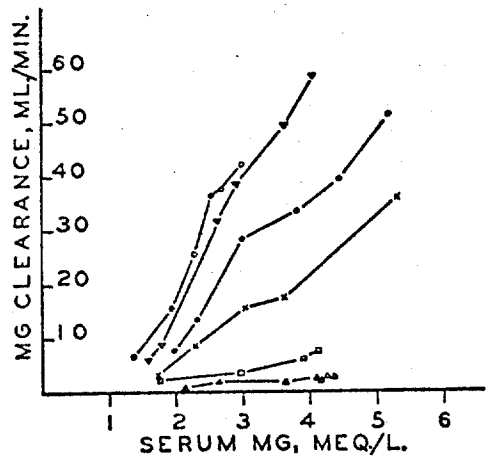

FIG. 2. THE RELATIONSHIP BETWEEN THE UNCORRECTED RENAL CLEARANCE OF MAGNESIUM AND THE SERUM CONCENTRATION OF MAGNESIUM AT VARIOUS LEVELS OF GLOMERULAR FILTRATION

Reading down from the top, the lines are as follows: ○—inulin clearance, 196 ml. per minute. Nonpregnant "normal" subject given intramuscular magnesium sulfate [Procedure (*c*)]. ▽—inulin clearance, 150 ml. per minute. Pregnant hypertensive patient given magnesium acetate infusion [Procedure (*a*)]. ●—inulin clearance, 88 ml. per minute. Nonpregnant "normal" subject given magnesium acetate infusion [Procedure (*a*)]. ×—inulin clearance, 57 ml. per minute. Pregnant hypertensive patient given magnesium acetate infusion [Procedure (*a*)]. □—inulin clearance, 25 ml. per minute. Pregnant patient with glomerulonephritis given constant infusions of magnesium sulfate [Procedure (*b*)]. △—pregnant patient in uremia. Inulin clearance not measured; urea clearance, 3 ml. per minute.

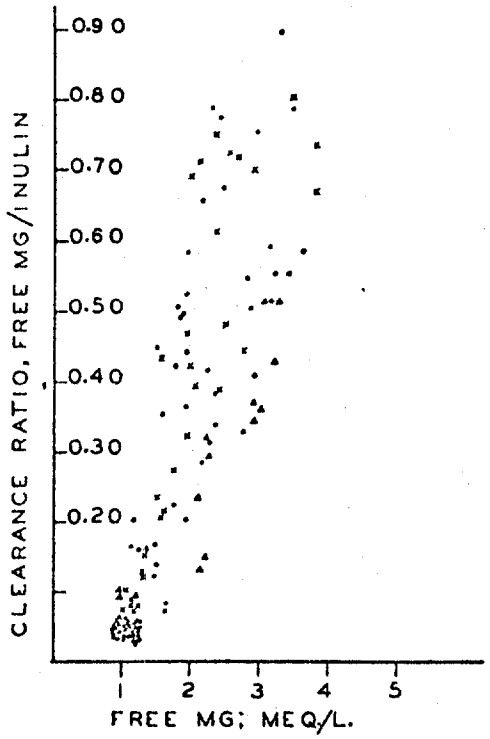

FIG. 3. THE RELATIONSHIP BETWEEN THE CLEARANCE RATIO OF "FILTRABLE" MAGNESIUM TO INULIN AND THE SERUM CONCENTRATION OF "FILTRABLE" MAGNESIUM

The inulin clearances ranged from 25 to 196 ml. per minute. ●, magnesium sulfate; ×, magnesium acetate; △, patients with glomerulonephritis.

higher filtered loads, while points below the line would represent increased tubular reabsorption. Except for the three abnormal cases, most of the points fall close enough to the line to suggest that the tubular reabsorption of magnesium was nearly constant over the range of filtered loads studied and that reabsorption was nearly maximal under "basal" conditions. When the control load was increased by less than 50 μEq. per minute, most of the points fell below the line. This would suggest that the tubular reabsorptive mechanism was not quite saturated before magnesium was injected.[2]

In the three aberrant cases the tubular reabsorption appears to have increased when the filtered load increased. In one of these, a preeclamptic, the control inulin clearance of 86 ml. per minute nearly doubled and became variable after magnesium sulfate was injected. In the other two the inulin clearance varied but little from the control values of 110 ml. per minute in the patient with preeclampsia and 170 ml. per minute in the one with essential hypertension.

Effect of magnesium administration upon calcium excretion

The usual effect of magnesium injection was to increase the urinary excretion of calcium. Roughly, the calcium excretion increased as the serum concentration of magnesium rose, as Fig-

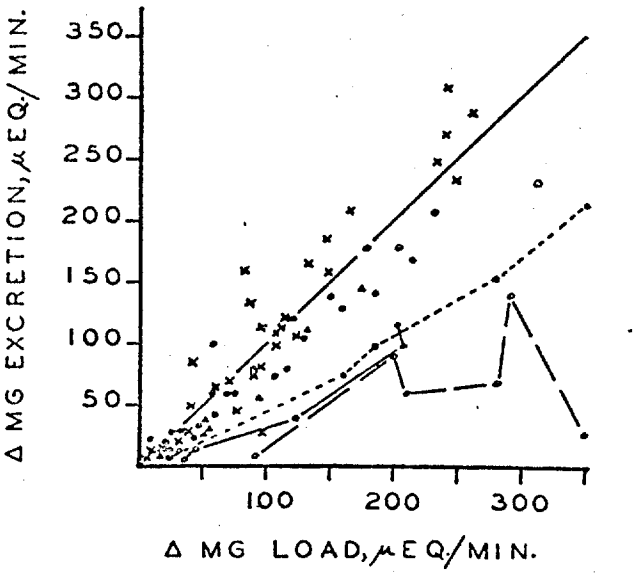

FIG. 4. THE RELATIONSHIP BETWEEN INCREMENTS IN MAGNESIUM EXCRETION AND INCREMENTS IN THE FILTERED LOAD OF MAGNESIUM

The points connected by the broken lines indicate observations in three abnormal and atypical patients. ×, normal; ●, hypertension; △, renal disease; ○, preeclampsia.

[2] In 43 observations the increments in filtered load exceeded 50 μEq. per minute. The line fitted to these points by the method of least squares had its intercept at −6, $S_a = 12.44$; this is not significantly different from a zero intercept ($t = 0.483$, $p > 0.5$). The slope of the line was 1.031, $S_b = 0.086$: this is not significantly different from a slope of 1 ($t = 0.36$, $p > 0.5$). Similarly, a line was fitted to the 23 points representing increments of less than 50 μEq. per minute in the filtered load. Its intercept was +7, $S_a = 5.4$, not significantly different from 0 ($t = 1.3$, $p = 0.2$). The slope was 0.414, $S_b = 0.2$. This is significantly different from 1 ($t = 2.93$, $p < 0.01$). Therefore the points for increments of less than 50 μEq. per minute do fall significantly below the line shown in the graph. The three aberrant cases have been excluded from this analysis.

ure 5 shows. Only seven observations were made at serum magnesium concentrations greater than 5 mEq. per liter and the average drop shown for calcium excretion at these high levels of serum magnesium probably is not real, for it was not seen in individual cases. The increases in calcium excretion following magnesium acetate were comparable to those following magnesium sulfate injections.

Calcium excretion was measured in 29 patients. Only 2 failed to show increased urinary loss of calcium after the injection of a magnesium salt. One of these had an inulin clearance of 25 ml. per minute (glomerulonephritis) and the other, with mild preeclampsia, had an inulin clearance of 110 ml. per minute. The calcium excretion in any 1 patient, other than these 2, was always maximal when the serum magnesium was at its highest level. In 12 cases, observations were made while the serum magnesium concentration first rose and later declined. In every such instance the calcium excretion decreased from its peak as the serum magnesium fell.

Effect of magnesium administration upon sodium and chloride excretions

Both magnesium acetate and magnesium sulfate injections increased the sodium and chloride excretions (Figure 5). Twenty-nine patients were studied in 100 clearance periods following magnesium injections. Sodium and chloride excretions were almost equimolar and the coefficient of correlation (r) between them was 0.80. The stimulation of sodium and chloride excretions seemed to depend upon a rising serum concentration of magnesium, for whenever the serum magnesium became nearly constant or fell the excretions dropped back toward, to, or below the control levels. Only one patient failed to show an augmentation in sodium and chloride excretion while the serum magnesium was rising; she had essential hypertension and in the control periods had high excretions (chloride, 760 μEq. and sodium, 722 μEq. per minute). Following the intramuscular injection of magnesium sulfate, the excretions fell to about one-third of the control values.

Figure 6 shows what happened to sodium and chloride excretions when the serum magnesium rose, then remained nearly constant, and then fell.

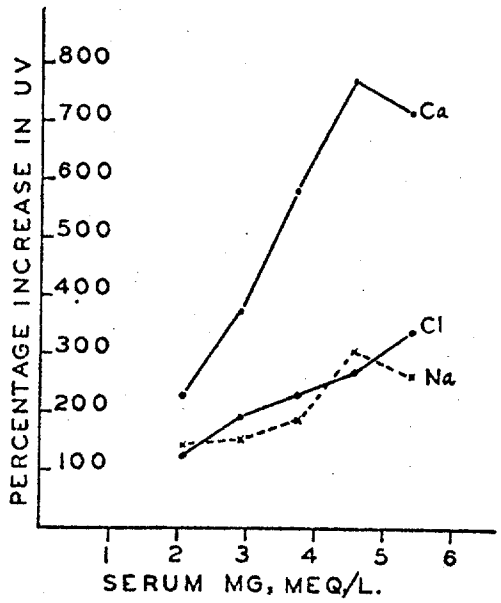

FIG. 5. THE STIMULATION OF CALCIUM, SODIUM AND CHLORIDE EXCRETIONS AND THEIR DEPENDENCE UPON SERUM CONCENTRATION OF MAGNESIUM

All patients, normal and abnormal, have been pooled.

The lines connect points representing consecutive observations. Sodium and chloride excretions rose sharply while the serum magnesium was increasing, dropped precipitously while the serum magnesium was essentially constant, and fell back toward, to, or below control levels when the serum magnesium began to decline. This was observed in all six cases in which Procedure (*d*) was used. Six of the patients studied with Procedure (*c*) had serum magnesium concentrations that became constant or fell during the periods in which sodium and chloride excretions were measured; all showed the same effect. Unfortunately, inulin clearances were not measured in any of these periods and nothing can be said about a possible relationship between changing filtration rates and changes in sodium excretion.

Effect of magnesium administration upon potassium excretion

Four of the six patients infused with magnesium acetate had sustained decreases in potassium excretion during the infusion. The two exceptions both had depressed inulin clearances. One, with glomerulonephritis, had a control inulin clearance of 52 ml. per minute and a sustained increase in

potassium excretion. The other, with long-standing and rather severe essential hypertension, had a control inulin clearance of 57 ml. per minute and a sustained increase (of less than 10 per cent) in potassium excretion.

Following the injection, or during the infusion, of magnesium sulfate only 8 of 22 subjects had sustained decreases in potassium excretion. Six had sustained increases and these six patients were represented in the nonpregnant, normal pregnant, preeclamptic and pregnant nephritic groups.

Excluding the two patients with glomerulonephritis, in whom potassium excretions were measured, and averaging all other observations, magnesium sulfate depressed potassium excretion by a mean of 11 per cent, but the excretion was actually increased in 39 per cent of the periods. The mean decrease in potassium excretion during magnesium acetate infusions was 25 per cent and increases were seen in only 2 of 29 periods.

No correlation could be discerned between changes in potassium excretion and the serum concentrations or direction of change in serum concentrations of magnesium.

A relation was sought between changes in sodium excretion and changes in potassium excretion, on the hypothesis that magnesium might interfere with the renal tubular exchange of potassium for sodium. However, no relation was found. When sodium excretion increased, potassium excretion might decrease, increase, or remain at the same level.

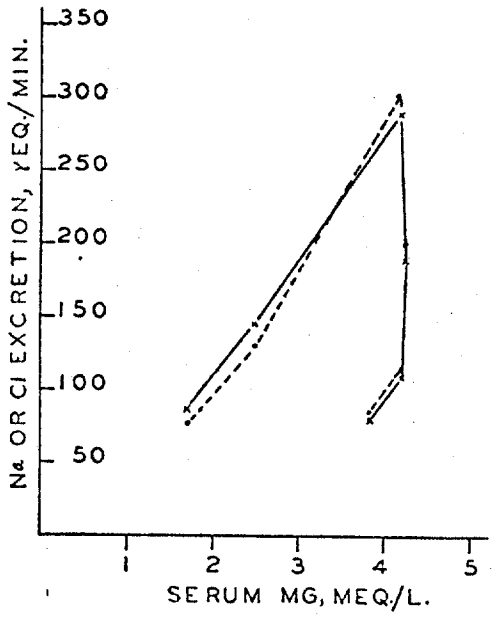

FIG. 6. SODIUM AND CHLORIDE EXCRETIONS RELATED TO THE TREND OF SERUM MAGNESIUM CONCENTRATION

The graph shows data for one representative case. The lines connect consecutive observations. Broken line denotes chloride excretion, solid line denotes sodium excretion.

DISCUSSION

The method used for the measurement of serum and urine magnesium concentrations was chosen because it gave us better recoveries of added magnesium than did the titan yellow method. Engbaek (16), citing earlier work of his own, wrote that the preliminary separation of calcium by oxalate precipitation does not interfere with the measurement of magnesium in normal serum. However, ". . . when the serum contains about 10 times the (Mg) value of the normal serum, the separation of calcium results in a loss of 4 to 8 per cent of the total magnesium." This results from the occlusion of magnesium oxalate in the calcium oxalate precipitate. This source of error was partially offset by the preliminary dilution of sera and urines expected to have high concentrations of magnesium.

Although several investigators (1–8) have shown that the renal clearance (or excretion) of magnesium increases after the injection of magnesium salts, no data have been published to show how the renal clearance of magnesium depends upon the serum magnesium concentration. (Barker, Clark and Elkinton (8) have published only an abstract of their systematic work on this.) The published clearance findings are summarized in Table III.

Figures 1, 2 and 3 indicate that the magnesium clearance increases as a roughly linear function of the serum magnesium concentration and that the attendant anion has but little effect. Magnesium acetate was chosen for comparison to the sulfate because the rapid utilization of acetate by the body would remove a large proportion of the anion, which presumably was replaced by bicarbonate. Thus the high clearance rate of such an anion as sulfate would not obligate the excretion of cations such as magnesium and a nearly "pure" effect of magnesium could be studied. Presumably the

TABLE III

Reported effects of magnesium loading upon renal excretion of magnesium

Author	Subjects	Control observations			Magnesium sulfate	After magnesium loading		
		Serum Mg	C_{Mg}*	C_{Mg}/C_I*		Serum Mg	C_{Mg}	C_{Mg}/C_I
		mEq./L.	*ml./min.*	*×100*		*mEq./L.*	*ml./min.*	*×100*
Schwartz, Smith and Winkler (2)	5 dogs		1–2		Intravenous		12–24	29–45†
Heller, Hammarsten and Stutzman (3)	6 men	1.49–1.73	3.98–6.75	2.9–5.3	1.02 mEq./min., I.V.	2.07–2.42	24–32	16–25
Womersley (4)	5 men	1.5–1.9		2.3–5.6	20 mEq., I.V.	2.3–2.8		16–22

* C_{Mg}, uncorrected magnesium clearance; C_I, inulin clearance.
† $C_{Mg}/C_{Creatinine}$.

acetate infusions resulted in the production of alkaline urines, which may have affected magnesium (and potassium) excretions.

The derivation of "filtrable" magnesium from the nomogram of Willis and Sunderman (10) assumes that the injected magnesium was promptly bound to plasma proteins to the same degree that slowly accumulated "endogenous" magnesium would be in the steady state. Hopkins, Howard and Eisenberg (17) added magnesium chloride to serum and found that in the range of 2.2 to 26.4 mg. per 100 ml. the amount of magnesium bound to proteins rose proportionately to the total serum concentration of magnesium. They found the same to be true for calcium, as did Terepka, Toribara and Dewey (18). Thus, binding sites are available and Copeland and Sunderman (9) found that magnesium proteinate acts as a dissociated salt, following the mass action law with a calculated pK of 1.77.

In the magnesium acetate infusion studies, the plasma pH may have increased somewhat. Possibly this could have affected the proportion of magnesium that was filtrable. Against this possibility is the fact that Hopkins, Howard and Eisenberg (17) found no effect of pH upon the filtrable fraction of calcium, in the range of pH 7.26 to 7.47; at higher pH levels, the filtrable fraction decreased. They also studied the effect of pH on the filtrability of magnesium, in the range of pH 7.28 to 7.46, and apparently found no effect (although this was not clearly stated so strongly).

In calculating the "corrected" clearances, as well as the filtered loads and tubular reabsorption of magnesium, the validity of the derivation of filtrable magnesium is crucial. If the injected magnesium is not bound to proteins in the same proportion as is the basal serum magnesium, then the derived data underestimate the filtered load and, therefore, the amount of magnesium reabsorbed by the tubules.

Hammarsten, Allgood and Smith (6) found that patients with advanced renal disease and azotemia had abnormally high ratios of "corrected" magnesium clearances to inulin clearances before magnesium was given. The triangles in Figure 3 show that this was true in our three cases of chronic glomerulonephritis with lesser degrees of renal impairment (inulin clearances from 25 to 60 ml. per minute). These investigators also noted that the clearance ratio did not increase so much in the nephritic patients after magnesium injection as it did in normal subjects. This is not so clear-cut in Figure 3, perhaps because of the relatively good residual function in our cases. The inulin clearance was not measured in the one patient with azotemia and a urea clearance of 3 ml. per minute, but the magnesium clearance increased only slightly after the injection of magnesium sulfate (triangles in Figure 2)—*i.e.*, the clearance ratio probably did not increase by much.

The increase in calcium excretion after the injection of magnesium salts confirms other investigations (1, 4) and in addition the present data show a good correlation between the serum concentration of magnesium and the increase in calcium loss; in individual subjects the correlation was almost linear. This was equally true for both rising and falling serum concentrations of magnesium, which is quite different from the

effect of magnesium upon the excretion of sodium and chloride. The acetate and sulfate salts were equally effective. This means that the calcium excretion was not obligated by sulfate excretion, as might be inferred from the quotation of Schwartz, Smith and Winkler (2) in the introduction. Perhaps the renal tubule cannot differentiate perfectly between calcium and magnesium, which may compete for reabsorption. No explanation is offered for the failure of magnesium to stimulate the excretion of calcium in 2 of the 29 patients studied.

The dependence of the stimulation of sodium and chloride excretions upon a rising concentration of serum magnesium explains the transitory effect noted by Womersley (4) and Pritchard (15), who gave single intravenous injections of magnesium sulfate, and the sustained effect reported by Heller, Hammarsten and Stutzman (3), who gave continuous infusions. Womersley did not find any stimulation of sodium or chloride excretion after magnesium lactate, although magnesium acetate in the present study did have such an effect. This discrepancy and the statement of Schwartz, Smith and Winkler (2) that magnesium sulfate injections almost completely repressed the excretion of chloride remain unresolved. Womersley (4) suggested that the enhanced excretion of sodium following magnesium sulfate might be obligated by the excretion of the sulfate ion. However, chloride and sodium excretions were almost equimolar in the present study (and in his own averages), and the augmentation in their excretions was as great after magnesium acetate as that following magnesium sulfate.

The stimulation of sodium excretion might be attributed to osmotic diuresis, but against this interpretation is the fact that the sodium excretion fell abruptly when the serum magnesium concentration attained a plateau (as in Figure 6) and the excretions of magnesium and calcium were essentially constant. Also, Womersley (4) did not find significant increases in the rate of excretion of total osmols following the injection of 20 mEq. of magnesium as the sulfate salt. No explanation can be offered for the peculiar observation that the sodium and chloride excretions were enhanced only so long as the serum magnesium concentration was rising.

Heller, Hammarsten and Stutzman (3), with six subjects, and Womersley (4), with five, reported significant decreases in potassium excretion after magnesium sulfate injections. Pritchard (15) found no significant change in seven women with preeclampsia. Only 12 of our 28 patients had sustained decreases, while 6 had sustained increases in potassium excretion after injection of magnesium salts.

Unfortunately, the pH of the urines was not measured but if they were alkaline after the acetate injections, as seems probable, one would expect potassium excretion to be enhanced rather than decreased. Yet the potassium excretion was decreased in 27 of the 29 periods during magnesium acetate infusion (omitting the patient with nephritis). Berliner, Kennedy and Orloff (19) have reviewed the evidence for competition between hydrogen and potassium ions for exchange with sodium ions in the tubular lumen: When the urine is alkaline, hydrogen ion secretion is suppressed and potassium secretion is augmented.

SUMMARY AND CONCLUSIONS

Some effects of parenteral magnesium acetate and sulfate upon renal excretion of electrolytes were studied in 34 "normal" and abnormal women, selected to give a wide range of inulin clearances.

The magnesium clearance increased as a roughly linear function of the serum magnesium concentration; the attendant anion—acetate or sulfate—had little or no distinguishable effect upon the excretion of magnesium.

The ratio of magnesium clearance, corrected by nomogram for protein binding of serum magnesium, to inulin clearance approached 1 at the highest levels of serum magnesium studied. Roughly, this ratio was a linear function of the serum "filtrable" magnesium.

It appears that the tubular reabsorption of magnesium may be nearly maximal under "basal" conditions. Increments in the filtered load of magnesium, over "basal" levels, are excreted almost completely. This conclusion is tentative, for there is some uncertainty as to the validity of the calculations.

The injection of magnesium salts was followed by augmented urinary excretion of calcium. The increase in calcium excretion bore a direct relation to the serum magnesium concentration.

Stimulation of sodium and chloride excretions was observed as long as the serum magnesium concentration was rising. When the serum magnesium became constant, or fell, the excretions of sodium and chloride dropped back toward, to, or below control levels.

The effect of magnesium upon potassium excretion varied from subject to subject.

ACKNOWLEDGMENTS

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The Experimental Action of Waters Containing Magnesium Chloride on the Intestine "in vivo".

by M. Chiray, L. Justin-Besancon and Ch. Debray

Bull. Acad. Nat. Med. 132: 76-78, 1948

The action of magnesium chloride waters on smooth fiber particularly on intestinal smooth fiber has already been studied considerably. Did not Fleig, as early as 1900, describe the decline of the intestinal tonus under the effect of the water of Chatel-Guyon? Later, one of us, with Professor Maurice Villaret[1] showed that adding water containing magnesium (Chatel-Guyon Gubler) to a bath of Tyrode solution, elicits a decline in tonus and an exaggeration of the amplitude of the longitudinal contractions, in the smooth fiber of isolated rabbit intestine. When the contraction of the isolated loop is weak the magnesium containing water causes a sharp elevation in its amplitude at the same time as an average decline of tonus. On the other hand, in an intestine undergoing strong contractions the action of Chatel-Guyon water is much less conclusive.

All these experiments have been conducted on the isolated intestine and not on the intact organ. In the present account, we have tried to specify the action of magnesium chloride on the intestine in vivo.

For this we turned our attention to the intenstine of the frog and the guinea pig. A cannula was introduced by a lateral opening without sectioning the intestine. The perfusion liquid was fed in by this

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[1] Villaret (Maurice) and Justin-Besancon (L.), Experimental hydrology, Paris 1933. Masson edit.

cannula. A second opening was made laterally, 2 or 3 cm away to allow the perfusion liquid to exit easily. By this means a short section of intestine, 2 or 3 cm, was studied. Consequently the vascular and nerve connections remained in their respective relative positions. We used Tyrode's solution for the frog perfusion. An electric drop counter, set up under our direction, made it possible to record the drops which crossed the section of intestine. In the frog the perfusion was carried out at a constant pressure of 10 to 15 cm of water and in the guinea pig at 25 to 30 cm. Naturally in these, the perfusion solution like the solution of magnesium chloride was kept at 38° during the entire passage through the tubing. The first few centimeters of the small intestine was studied in the frog. In the guinea pig our research was done on the end of the ileum and the ileocecal valve. Moreover, we note that before the experiment the frogs medulla was destroyed and the guinea pig was lightly anesthetized with ether.

Under these conditions, the flow of the drops of physiological perfusion liquid per minute is constant. Occasionally, especially in the guinea pig, brief and rhythmic variations which correspond to the passage of peristaltic waves are seen. The flow of drops stops for a few seconds during the passage of the bands of contraction, then starts up again. If care is taken to make measurements at a sufficiently long time, for example to count the drops which pass during one or more minutes, and if one puts these figures on a graph, these transitory contractions only show up a little or not at all on the curve.

When the physiological perfusion solution is replaced by water containing magnesium chloride[2], a considerable slowing down in the flow

[2] We used Chatel-Guyon water, transported from the Gubler spring.

of the drops is recorded. It is as if in the frog where for example the flow alters from 50-55 per minute to about 15, it would be a 70 percent decrease. This slowing down of the flow is established in a few minutes and remains as long as the perfusion of water containing magnesium chloride lasts. It disappears almost instantaneously as soon as the Chatel-Guyon water is replaced by physiological solution and, after a few drops, the flow returns to its former rate.

In the guinea pig, the results are the same, except that the flow is much less slowed down, the number of drops reduced, for example, from 38 to 28 and the return to the former flow takes several minutes to establish.

Thus these curves show directly the contractions in vivo of the smooth fiber of the intestine under the influence of magnesium chloride. If one carefully examines the movements of the intestine thus perfused, one further ascertains that this contraction is visible and that it traverses the segment of the intestine studied in the form of a peristaltic wave. It is therefore not a question of intestinal contracture but of the amplification of normal contractile waves.

This in vivo effect is related to what happens in man when magnesium chloride has a classical laxative action. Furthermore, we know that Chatel-Guyon water taken in very strong doses causes diarrhea. This is what happened formerly when sick people took considerable doses daily, going from 1500 cc to still larger amounts. This action of the water conveys its excitomotor effect on the intestine to the liver and its excitosecretory ability to the digestive tract. This old practice of

using excessive doses is moreover not being continued, as one knows, except for serious problems such as a congestive state of the liver, kidney blockage in brightics, gastric heaviness, and also the onset of the crisis in hepatic colic or of appendicitis with certain patients. So, these strong doses of magnesium chloride water have been completely abandoned.

The effects in man are entirely different, with present average doses of about a few hundred grams, the therapeutic dose of Chatel-Guyon, the waters regulate intestinal function. The decontracting role in spasmodics which is on the contrary contracting in atonics has been well known by the clinicians of Chatel-Guyon since Baraduc and Foucauld. It would also be interesting in this respect to study experimentally the effect of magnesium chloride containing water not only on a normal intestine but on an intestine contracted by means of various pharmacodynamic agents.

Summary

The perfusion with water containing magnesium chloride of a small section of the small intestine of the frog and the guinea pig in vivo causes a reduction in the flow of the perfusate by means of the contraction of the smooth fibers of the intestine. This contraction is due to a strong exaggeration of the normal peristaltic waves.

Bull Acad Nat Med, 132 : 76-78, Feb '48

Le gérant de la société de désinsectisation avait doublement enfreint le règlement : d'abord, il avait fait une désinsectisation par l'acide cyanhydrique sans avoir prévenu, quarante-huit heures à l'avance, le Directeur départemental de la Santé ; ensuite, il n'avait pas pratiqué, après aération, l'épreuve des petits animaux pour détecter la présence d'acide cyanhydrique.

En outre, il était déraisonnable d'employer, pour désinsectiser une maison, l'acide cyanhydrique, gaz particulièrement dangereux. La chloropicrine aurait donné des résultats comparables et aurait été pratiquement sans danger, en raison de son odeur et de son effet lacrymogène ; les locataires auraient constaté que la chloropicrine n'avait pas été totalement évacuée et un accident mortel aurait été évité.

En ce qui concerne la dératisation, les gaz toxiques, notamment la chloropicrine, sont parfois employés. L'opération est délicate ; il est nécessaire d'obturer au préalable tous les trous de rats. Or, ces derniers sont parfois nombreux dans les caves et on risque de ne pas les boucher tous. Dans ce cas, le gaz toxique gagne les caves voisines, un accident est donc possible. Pour éviter cette éventualité, il est prescrit, depuis 1946, de ne faire ces opérations que dans des immeubles isolés.

A la Commission d'épidémiologie du Conseil Supérieur d'Hygiène, de nombreux membres ont souligné les dangers de l'acide cyanhydrique ; l'interdiction de ce gaz a été demandée dans la désinsectisation et la dératisation des maisons.

Aucune modification n'a été proposée en ce qui concerne les usagers agricoles et la Marine marchande.

Le Conseil a demandé en outre l'interdiction de l'emploi de l'oxyde d'éthylène et du bromure de méthyle, dont les dangers ont été maintes fois soulignés. Par contre, il a paru nécessaire de maintenir l'autorisation de l'emploi de la chloropicrine qui est beaucoup moins dangereuse, et qui, comme l'a montré le professeur Gabriel Bertrand, donne d'excellents résultats.

L'Académie de Médecine pourrait s'associer au Conseil Supérieur d'Hygiène en adoptant le vœu suivant :

« L'Académie de Médecine, en raison des accidents mortels provoqués par l'acide cyanhydrique, le bromure d'éthyle et l'oxyde d'éthylène, demande que soit interdit l'emploi de ces gaz toxiques dans la désinsectisation et la dératisation des locaux d'habitation.

« Elle estime, par contre, que l'emploi de la chloropicrine, produit très actif et beaucoup moins dangereux en pratique, doit être maintenu. »

M. Fabre s'associe aux conclusions de MM. Tanon et Poyer. Les dispositions réglementaires prises en vue de l'emploi des fumigants pour la désinsectisation de locaux sont suffisamment précises et sévères pour éviter tout accident. Malheureusement l'exemple cité par les auteurs et confirmé par plusieurs cas analogues apporte la preuve qu'elles ne sont pas toujours suivies, ce qui est éminemment regrettable si l'on veut assurer une protection satisfaisante de la santé.

L'Académie, à l'unanimité, adopte le vœu ci-dessus.

Action expérimentale des eaux chlorurées magnésiennes sur l'intestin « in vivo »,

par MM. **M. Chiray, L. Justin-Besançon** et **Ch. Dobray.**

L'action des eaux chlorurées magnésiennes sur la fibre lisse et en particulier sur la fibre lisse intestinale, a déjà été fort étudiée. Dès 1880, Fleig n'avait-il pas signalé la chute du tonus intestinal sous l'action de l'eau de Châtel-Guyon ? Plus

laud, l'un de nous, avec le professeur Maurice Villaret (1), a montré que l'addition d'eau magnésienne (Châtel-Guyon-*Gubler*) au bain de Tyrode, provoque, sur la fibre lisse de l'intestin isolé du lapin, une chute du tonus et une exagération de l'amplitude des contractions longitudinales. Quand les contractions de l'anse isolée sont faibles, l'eau magnésienne détermine l'élévation brutale de leur amplitude, en même temps qu'une chute moyenne du tonus. Au contraire, sur un intestin à contractions fortes, l'action de l'eau de Châtel-Guyon est beaucoup moins démonstrative.

Toutes ces expériences ont été conduites sur *l'intestin isolé* et non sur l'organe en place. Dans la note actuelle, nous avons au contraire tenté de préciser l'action de l'eau chlorurée magnésienne sur un intestin *in vivo*.

Nous nous sommes adressés pour cela à l'intestin de grenouille et de cobaye. Sans sectionner l'intestin, une canule y est introduite par une ouverture latérale et par cette canule arrive le liquide de perfusion. A 2 ou 3 centimètres en aval, une seconde ouverture est pratiquée latéralement pour permettre au liquide de perfusion de s'échapper facilement. Ainsi est étudié un court fragment d'intestin de 2 ou 3 centimètres. Les connexions vasculaires et nerveuses restent donc relativement respectées. Pour la perfusion nous avons employé chez la grenouille le liquide de Tyrode. Un compte-gouttes électrique, établi sur nos indications, permet d'enregistrer les gouttes qui traversent le fragment intestinal. La perfusion fonctionne sous une pression constante de 10 à 15 centimètres d'eau chez la grenouille, de 25 à 30 centimètres chez le cobaye. Naturellement, chez celui-ci, l'eau de perfusion comme l'eau chlorurée magnésienne est maintenue à 38° pendant tout le trajet dans le caoutchouc. Pour la grenouille, ce sont les premiers centimètres du grêle qui ont été étudiés. Pour le cobaye, c'est la fin de l'iléon et la valvule iléo-caecale qui ont servi à nos recherches. Signalons encore que la grenouille a eu avant l'expérience la moelle détruite et que le cobaye est légèrement anesthésié à l'éther.

Dans ces conditions, avec le liquide de perfusion physiologique, le débit des gouttes par minute est constant. On observe parfois, surtout chez le cobaye, de brèves et rythmiques variations qui correspondent au passage des ondes péristaltiques. L'écoulement des gouttes s'arrête quelques secondes au passage de l'anneau de contraction, puis reprend. Si on prend la précaution de faire des mesures sur un temps suffisamment long, par exemple de compter les gouttes qui passent pendant une minute ou plus, et si l'on porte ces chiffres sur un tracé, ces resserrements transitoires ne se manifestent que très peu ou pas sur la courbe.

Quand on remplace le liquide physiologique de perfusion par de l'eau chlorurée magnésienne (2), on constate immédiatement un ralentissement considérable dans la chute des gouttes. C'est ainsi que, chez la grenouille, le débit passe par exemple de L-LV gouttes par minute à XV environ, soit une diminution de 70 p. 100. Ce ralentissement du flux s'installe en quelques minutes et persiste tant que dure la perfusion d'eau chlorurée magnésienne. Il disparaît presque instantanément dès que l'on remplace l'eau de Châtel-Guyon par le liquide physiologique et, à quelques gouttes près, le débit devient ce qu'il était auparavant.

Chez le cobaye, les résultats sont identiques avec cette différence toutefois que le ralentissement du débit est beaucoup moindre, le nombre des gouttes passant par exemple, de XXXVIII à XXVIII et que le retour au débit antérieur prend quelques minutes pour s'établir.

Ces courbes objectivent donc de la manière la plus nette la contraction des fibres lisses dans l'intestin *in vivo* sous l'effet de l'eau chlorurée magnésienne.

Quand on examine avec soin les mouvements de l'intestin ainsi perfusé, on

(1) Villaret (Maurice) et Justin-Besançon (L.), *Hydrologie expérimentale*. Paris 1933. Masson, édit.

(2) Nous avons utilisé l'eau de Châtel-Guyon, source *Gubler* transportée.

constate d'ailleurs que cette contraction est visible et qu'elle parcourt le segment intestinal étudié sous la forme d'ondes péristaltiques. *Il ne s'agit donc pas de contracture intestinale, mais de l'exagération des ondes contractiles normales.*

Cet effet *in vivo* est à rapprocher de ce qui se passe chez l'homme où le chlorure de magnésium a une action laxative classique. D'ailleurs, on sait que l'eau de Châtel-Guyon, prise à de trop fortes doses, détermine la diarrhée. C'est ce qui se passait jadis quand les malades absorbaient des doses journalières considérables, allant de 1.500 c. c. à des quantités plus grandes encore. Cette action de l'eau traduit à la fois son effet excito-moteur sur l'intestin et son pouvoir excito-sécrétoire sur le tube digestif. Les pratiques anciennes utilisant des doses excessives n'allaient d'ailleurs pas, on le sait, sans de sérieux inconvénients tels qu'effet congestif du foie, blocage des reins chez les brightiques, pesanteurs gastriques et même déclenchement de crises de coliques hépatiques ou d'appendicite chez certains patients. Aussi ces fortes doses d'eau chlorurée magnésienne ont-elles été complètement abandonnées.

Avec les doses moyennes actuelles de quelques centaines de grammes, doses thérapeutiques à Châtel-Guyon, les effets sur l'homme sont tout différents. Les eaux régularisent le fonctionnement intestinal. Ce rôle décontractant chez les spasmodiques et, au contraire, contractant chez les atones est bien connu des cliniciens de Châtel-Guyon depuis Baradue et Foucault. Aussi serait-il peut être intéressant d'étudier à cet égard l'action expérimentale des eaux chlorurées magnésiennes, non plus sur un intestin normal, mais sur un intestin contracturé, par divers agents pharmacodynamiques.

Résumé. — Chez la grenouille et le cobaye la perfusion d'un fragment d'intestin grêle *in vitro* avec l'eau chlorurée magnésienne détermine une diminution du flux de perfusion par contraction des fibres lisses de l'intestin. Cette contraction est due à une forte exagération des ondes péristaltiques normales.

A propos de la communication de M. Danielopolu sur la « possibilité d'intercepter les vaso-constricteurs des membres supérieurs sans toucher au ganglion étoilé »,

par M. **J. Delmas.**

Dans une communication à l'Académie de Médecine en date du 11 octobre 1947, le professeur Danielopolu, reprenant la question toujours d'actualité et toujours controversée de l'interruption des vaso-constricteurs du membre supérieur, fait une critique pertinente de la stellectomie, surtout si elle est accompagnée de la section des trois premiers ganglions thoraciques. Il insiste avec raison sur les troubles tardifs et parfois très graves que cette intervention peut entraîner dans le fonctionnement du myocarde et des coronaires puisqu'aussi bien ces opérations interceptent et les nerfs cardio-accélérateurs et les nerfs vaso-dilatateurs coronariens.

La méthode qu'il propose a donc pour but d'intercepter les vaso-constricteurs du membre supérieur sans toucher aux nerfs cardiaques et coronariens. Mais on peut se demander si, ainsi privée des inconvénients majeurs des méthodes précédentes, sa technique est pour autant la plus indiquée s'agissant d'interrompre les seules voies vaso-constrictives du membre supérieur. Les critiques et les suggestions que nous exposons dans cette courte notice ne s'appuient sur aucun document clinique ou expérimental. Elles sont basées uniquement sur les dernières acquisitions anatomiques dans la systématisation du sympathique. Elles nous paraissent cependant et à ce seul titre mériter d'être proposées au contrôle des physiologistes et des chirurgiens.

Tout d'abord il n'est pas tenu compte dans la communication de M. Danielo-

polu de l'espèce de *noli me tangere* que les auteurs récents opposent à la section des fibres post ganglionnaires en s'appuyant sur la sensibilisation extrême à l'adrénaline que ces sections font courir au territoire enervé. Ensuite, M. Danielopolu continue à accepter que les filets vaso-constricteurs du membre supérieur soient exclusivement des segments médullaires compris entre D 3 et D 11 (théories de Langberg et de Bedford). Cette conception systématique paraît difficilement acceptable aujourd'hui alors qu'elle s'applique à l'innervation d'un territoire somatique. Pour ces territoires les N. sympathiques, qu'ils soient vaso-moteurs, pilo-moteurs, sudoripares, obéissent à la loi de la métamérisation et les travaux de Von Mihalick et de Laruelle en ont donné la démonstration histologique comme ceux de Guerrier la démonstration macroscopique. Les racines du plexus brachial s'échelonnant entre C 5 et D 1 il était sage de rechercher si des fibres pré-ganglionnaires répondant à ces troncs nerveux n'empruntaient pas ces mêmes troncs avant d'aller faire synapse avec le neurone post-ganglionnaire dans des masses ganglionnaires qui leur seraient propres. C'est ce qu'ont démontré les délicates dissections effectuées dans notre laboratoire. Pour en comprendre l'exposé, il faut se rappeler qu'au niveau du cou les ganglions cervical supérieur et moyen ont surtout la valeur de masses prévisérales et qu'il doit donc exister dans le cou et la partie supérieure du thorax une chaine latéro-vertébrale type, dont les masses ganglionnaires ne serviraient de relai qu'aux voies somatiques par opposition aux viscérales.

Nous avons trouvé à la face postérieure de l'artère vertébrale et dans les deux premiers espaces intercostaux de petits amas ganglionnaires bien individualisés réunis par un connectif et dont l'ensemble constitue une chaine sympathique cervicale profonde. On doit admettre que la portion postéro-externe du stellaire appartient elle aussi à ces centres somatiques et que seule sa grosse portion antéro-interne représente un centre viscéral.

Ces notions une fois acquises nous avons pu trouver les rameaux communiquants blancs très courts qui, dans le canal vertébral reliaient les troncs du plexus brachial aux petits ganglions précités inclus dans le nerf vertébral classique comme aussi ceux qui se jetaient du 8e tronc cervical, du 1er et du 2e dorsal dans le segment postérieur du stellaire et dans deux petites masses ganglionnaires cervico-intercostales. Si donc on admet la conception de l'émergence métamérique des rameaux communicants blancs (fibres pré-ganglionnaires) et la nécessité de ne sectionner que ces fibres, mais toutes ces fibres, on conclura que l'interruption de la totalité des vaso constricteurs pré ganglionnaires du membre supérieur n'a jamais encore été réalisée et qu'elle ne pourrait l'être qu'au prix de difficultés techniques considérables sinon irréalisables sur le vivant. Ces difficultés résultent de la brièveté de ces R. C. B., de leur voisinage immédiat avec le R. C. G., de leur situation profonde dans le canal transversaire au milieu d'un lacis nerveux, veineux et fibreux extrêmement dense.

Ainsi s'expliquent les échecs rares, mais indéniables, les résultats incomplets ou temporaires des interventions réalisées jusqu'à ce jour et la nécessité de s'approcher tous les jours davantage de la rigueur anatomique aussi indispensable dans le domaine sympathique que dans le domaine cérébro spinal.

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TESTS

Angular rotation. Determine in a 100-mm. tube as directed under *Optical Rotation*, page 939.

Refractive index, page 945. Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in alcohol. Proceed as directed in the general method, page 899. One ml. dissolves in 4 ml. of 90 percent alcohol.

Specific gravity. Determine by any reliable method (see page 5).

Packaging and storage. Store in full, tight, glass, tin-lined, or other suitably lined containers in a cool place protected from light.

Labeling. Label mace oil to indicate whether it is the East Indian or West Indian type.

Functional use in foods. Flavoring agent.

MAGNESIUM CARBONATE

DESCRIPTION

Magnesium carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate. It occurs as light, white, friable masses, or as a bulky, white powder. It is odorless, and is stable in air. It is practically insoluble in water, to which, however, it imparts a slightly alkaline reaction. It is insoluble in alcohol, but is dissolved by dilute acids with effervescence. When treated with diluted hydrochloric acid T.S., it dissolves with effervescence and the resulting solution gives positive tests for *Magnesium*, page 927.

SPECIFICATIONS

Assay. The equivalent of not less than 40.0 percent and not more than 43.5 percent of MgO.

Limits of Impurities

Acid-insoluble substances. Not more than 500 parts per million (0.05 percent).

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Calcium oxide. Not more than 0.6 percent.

Heavy metals (as Pb). Not more than 30 parts per million (0.003 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Soluble salts. Not more than 1 percent.

TESTS

Assay. Dissolve about 1 gram, accurately weighed, in 30.0 ml. of 1 *N* sulfuric acid, add methyl orange T.S., and titrate the excess acid with

1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed, deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the weight of the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the magnesium oxide present. Each ml. of 1 *N* sulfuric acid is equivalent to 20.16 mg. of MgO and to 28.04 mg. of CaO.

Acid-insoluble substances. Mix 5.0 grams with 75 ml. of water, add hydrochloric acid in small portions, with agitation, until no more of the sample dissolves, and boil for 5 minutes. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool, and weigh.

Arsenic. A solution of 1 gram in 10 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Arsenic Test*, page 865.

Calcium oxide. Dissolve about 1 gram, accurately weighed, in a mixture of 3 ml. of sulfuric acid and 22 ml. of water. Add 50 ml. of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a Gooch crucible containing an asbestos mat that previously has been washed with diluted sulfuric acid T.S., water, and alcohol, and ignited and weighed. Wash the crystals on the mat several times with a mixture of 2 volumes of alcohol and 1 volume of diluted sulfuric acid T.S. Ignite the crucible and contents at a dull red heat, cool, and weigh. The weight of calcium sulfate so obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

Heavy metals. Dissolve 667 mg. in 10 ml. of diluted hydrochloric acid T.S., and evaporate the solution to dryness on a steam bath. Toward the end of the evaporation stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue in 20 ml. of water, and evaporate to dryness in the same manner as before. Redissolve the residue in 25 ml. of water, and filter if necessary. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 10 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Soluble salts. Mix 2.0 grams with 100 ml. of a mixture of equal volumes of *n*-propyl alcohol and water. Heat the mixture to the boiling point with constant stirring, cool to room temperature, add water to make 100 ml., and filter. Evaporate 50 ml. of the filtrate on a steam bath to dryness, and dry at 105° for 1 hour. The weight of the residue does not exceed 10 mg.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Alkali; drying agent; color-retention agent; anticaking agent; carrier.

MAGNESIUM CHLORIDE

$MgCl_2.6H_2O$ Mol. wt. 203.30

DESCRIPTION

Colorless, odorless flakes or crystals. It is very deliquescent. It is very soluble in water and freely soluble in alcohol. A 1 in 10 solution gives positive tests for *Magnesium*, page 927, and for *Chloride*, page 926.

SPECIFICATIONS

Assay. Not less than 99.0 percent and not more than the equivalent of 105.0 percent of $MgCl_2.6H_2O$.

Limits of Impurities

Ammonium. Not more than 50 parts per million (0.005 percent).

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).

Sulfate. Not more than 200 parts per million (0.02 percent).

TESTS

Assay. Dissolve about 450 mg., accurately weighed, in 25 ml. of water, add 5 ml. of ammonia-ammonium chloride buffer T.S. and 0.1 ml. of eriochrome black T.S., and titrate with 0.05 *M* disodium ethylenediaminetetraacetate until the solution is blue in color. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 10.16 mg. of $MgCl_2.6H_2O$.

Ammonium. Dissolve 1 gram in 90 ml. of water, and slowly add 10 ml. of a freshly boiled and cooled solution of sodium hydroxide (1 in 10). Allow to settle, then decant 20 ml. of the supernatant liquid into a color comparison tube, dilute to 50 ml. with water, and add 2 ml. of Nessler's reagent. Any color does not exceed that produced by 10 mcg. of ammonium (NH_4) ion in 48 ml. of water and 2 ml. of the sodium hydroxide solution.

Arsenic. A solution of 1 gram in 35 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Sulfate, page 879. Any turbidity produced by a 1-gram sample does not exceed that shown in a control containing 200 mcg. of sulfate (SO_4).

Packaging and storage. Store in tight containers.

Functional use in foods. Color-retention agent; firming agent.

MAGNESIUM HYDROXIDE

$Mg(OH)_2$ Mol. wt. 58.32

DESCRIPTION

A white, bulky powder. It dissolves in dilute acids, but is practically insoluble in water and in alcohol. A 1 in 20 solution in diluted hydrochloric acid T.S. gives positive tests for *Magnesium*, page 927.

SPECIFICATIONS

Assay. Not less than 95.0 percent of $Mg(OH)_2$ after drying.

Loss on ignition. Between 30.0 percent and 33.0 percent.

Limits of Impurities

Alkalies (Free) and soluble salts. Passes test.
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Calcium oxide. Not more than 1 percent.
Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).
Lead. Not more than 10 parts per million (0.001 percent).
Loss on drying. Not more than 2 percent.

TESTS

Assay. Transfer about 400 mg., previously dried at 105° for 2 hours and accurately weighed, into an Erlenmeyer flask. Add 25.0 ml. of 1 *N* sulfuric acid, and, after solution is complete, add methyl red T.S. and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed, deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the $Mg(OH)_2$ in the sample of magnesium hydroxide taken. Each ml. of 1 *N* sulfuric acid is equivalent to 29.16 mg. of $Mg(OH)_2$ and to 28.04 mg. of CaO.

Loss on ignition. Transfer about 500 mg., accurately weighed, to a tared platinum crucible, and ignite, increasing the heat gradually, to constant weight.

Alkalies (Free) and soluble salts. Boil 2 grams with 100 ml. of water for 5 minutes in a covered beaker, then filter while hot. Titrate 50 ml. of the cooled filtrate with 0.1 *N* sulfuric acid, using methyl red T.S. as the indicator. Not more than 2 ml. of the acid is consumed. Evaporate 25 ml. of the filtrate to dryness, and dry at 105° for 3 hours. Not more than 10 mg. of residue remains.

Arsenic. A solution of 1 gram in 25 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Arsenic Test*, page 865.

Calcium Oxide. Dissolve about 500 mg., accurately weighed, in a mixture of 3 ml. of sulfuric acid and 22 ml. of water. Add 50 ml. of alcohol, and allow the mixture to stand overnight. Warm the mixture to about 50°, if necessary, to dissolve any crystals of magnesium sulfate, and filter through a Gooch crucible containing an asbestos mat which has been previously washed with diluted sulfuric acid T.S., water, and alcohol, and ignited. Wash the crystals on the mat several times with a mixture of 3 volumes of alcohol and 1 volume of water. Ignite the crucible and contents at a dull red heat, cool, and weigh. The weight of calcium sulfate thus obtained, multiplied by 0.4119, gives the equivalent of calcium oxide (CaO).

Heavy metals. Dissolve 1 gram in 10 ml. of diluted hydrochloric acid T.S., and evaporate to dryness on a steam bath. Toward the end of the evaporation, stir the residue frequently, disintegrate it to obtain a dry powder, dissolve the powder in 20 ml. of water, and filter. A 10-ml. portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 20 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Loss on drying, page 931. Dry at 105° for 2 hours.

Packaging and storage. Store in tight containers.

Functional use in foods. Alkali; drying agent; color-retention agent.

MAGNESIUM OXIDE

MgO Mol. wt. 40.30

DESCRIPTION

A very bulky, white powder known as light magnesium oxide or a relatively dense, white powder known as heavy magnesium oxide. Five grams of light magnesium oxide occupy a volume of approximately 40 to 50 ml., while 5 grams of heavy magnesium oxide occupy a volume of approximately 10 to 20 ml. It is practically insoluble in water and is insoluble in alcohol. It is soluble in dilute acids. A solution of magnesium oxide in diluted hydrochloric acid T.S. gives positive tests for *Magnesium*, page 927.

SPECIFICATIONS

Assay. Not less than 96.0 percent of MgO after ignition.

Limits of Impurities

Acid-insoluble substances. Not more than 0.1 percent.
Alkalies (free) and soluble salts. Passes test.
Arsenic (as As). Not more than 3 parts per million (0.0003 percent).
Calcium oxide. Not more than 1.5 percent.

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Loss on ignition. Not more than 10 percent.

TESTS

Assay. Ignite about 500 mg. to constant weight at 800° to 825° in a tared platinum crucible, weigh the residue accurately, dissolve it in 30.0 ml. of 1 *N* sulfuric acid, boil gently to remove any carbon dioxide, cool, add methyl orange T.S., and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the magnesium oxide taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the MgO in the portion of magnesium oxide taken. Each ml. of 1 *N* sulfuric acid is equivalent to 20.15 mg. of MgO and to 28.04 mg. of CaO.

Acid-insoluble substances. Mix 2 grams with 75 ml. of water, add hydrochloric acid in small portions, with agitation, until no more dissolves, and boil for 5 minutes. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool, and weigh.

Alkalies (free) and soluble salts. Boil 2 grams with 100 ml. of water for 5 minutes in a covered beaker, and filter while hot. Add methyl red T.S., and titrate 50 ml. of the cooled filtrate with 0.1 *N* sulfuric acid. Not more than 2 ml. of the acid is consumed. Evaporate 25 ml. of the filtrate to dryness, and dry at 105° for 1 hour. Not more than 10 mg. of residue remains.

Arsenic. A solution of 1 gram in 20 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Arsenic Test*, page 865.

Calcium Oxide. Dissolve about 400 mg., accurately weighed, in a mixture of 3 ml. of sulfuric acid and 22 ml. of water. Add 50 ml. of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a Gooch crucible containing an asbestos mat that previously has been washed with diluted sulfuric acid T.S., water, and alcohol, and ignited and weighed. Wash the crystals on the mat several times with a mixture of 2 volumes of alcohol and 1 volume of diluted sulfuric acid T.S. Ignite the crucible and contents at a dull red heat, cool, and weigh. The weight of calcium sulfate obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

Heavy metals. Dissolve 500 mg. in 20 ml. of diluted hydrochloric acid T.S., and evaporate the solution to dryness on a steam bath. Toward the end of the evaporation stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue

in 20 ml. of water and evaporate to dryness in the same manner as before. Redissolve the residue in 20 ml. of water and filter if necessary. This solution meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A solution of 1 gram in 20 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Loss on ignition. Weigh accurately about 500 mg. in a tared covered platinum crucible. Ignite at between 800° and 825° for 15 minutes, cool and weigh.

Packaging and storage. Store in tight containers.

Labeling. Label magnesium oxide to indicate whether it is light magnesium oxide or heavy magnesium oxide.

Functional use in foods. Alkali; neutralizer.

MAGNESIUM PHOSPHATE, DIBASIC

Dimagnesium Phosphate

$MgHPO_4 . 3H_2O$ Mol. wt. 174.33

DESCRIPTION

A white, odorless crystalline powder. It is slightly soluble in water and insoluble in alcohol, but is soluble in dilute acids.

IDENTIFICATION

A. Dissolve about 200 mg. in 10 ml. of diluted nitric acid T.S. and add, dropwise, ammonium molybdate T.S. A greenish yellow precipitate of ammonium phosphomolybdate forms which is soluble in ammonia T.S.

B. Dissolve 100 mg. in 0.5 ml. of diluted acetic acid T.S. and 20 ml. of water. Add 1 ml. of ferric chloride T.S., let stand for 5 minutes, and filter. The filtrate gives a positive test for *Magnesium*, page 927.

SPECIFICATIONS

Assay. Not less than 96.0 percent of $Mg_2P_2O_7$, calculated on the ignited basis.

Loss on ignition. Between 29 and 36 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 10 parts per million (0.001 percent)

Heavy metals (as Pb). Not more than 30 parts per million (0.0[illegible] percent).

Lead. Not more than 5 parts per million (0.0005 percent).

TESTS

Assay. Weigh accurately about 500 mg. of the residue obtained in the test for *Loss on ignition*, dissolve it in a mixture of 50 ml. of water and 1 ml. of hydrochloric acid, dilute to 100.0 ml. with water, and mix. Transfer 50.0 ml. of this solution into a 250-ml. Erlenmeyer flask, add 10 ml. of ammonia-ammonium chloride buffer T.S. and 12 drops of eriochrome black T.S., and titrate with 0.1 *M* disodium ethylenediaminetetraacetate until the wine-red color changes to pure blue. Each ml. of 0.1 *M* disodium ethylenediaminetetraacetate is equivalent to 22.25 mg. of $Mg_2P_2O_7$.

Loss on ignition. Weigh accurately about 1 gram, and ignite, preferably in a muffle furnace, at 800° ± 25° to constant weight.

Arsenic. A solution of 1 gram in 5 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Arsenic Test*, page 865.

Fluoride. Transfer 5.0 grams of the sample into a 200-ml. distilling flask connected with a condenser and carrying a thermometer and a dropping funnel equipped with a stopcock. Dissolve the sample in 25 ml. of dilute sulfuric acid (1 in 4), add 6 glass beads, and connect the apparatus for distillation, using a 600-ml. beaker to collect the distillate. Add 40 ml. of the dilute sulfuric acid to the flask through the dropping funnel, then fill the funnel with water, heat the solution to boiling, and continue heating until the temperature reaches 165°. Adjust the stopcock of the dropping funnel so that the temperature is maintained at 165° ± 5°, and continue the distillation until about 300 ml. has been collected. Rinse the condenser and condenser arm with water, collecting the rinsings in the beaker. Add sodium hydroxide T.S. to the distillate to make it alkaline to litmus paper, and then add 5 ml. in excess. Add 5 ml. of 30 percent hydrogen peroxide and 6 glass beads to the beaker, boil until a volume of about 30 ml. is reached, and cool. Transfer the condensed distillate, including the glass beads, into a 125-ml. distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Add 30 ml. of perchloric acid, and continue as directed under the *Fluoride Limit Test, Method I*, page 917, beginning with "Connect a small dropping funnel or a steam generator to the capillary tube."

Heavy metals, page 920. Suspend 1.33 grams in 20 ml. of water, and add hydrochloric acid, dropwise, until the sample just dissolves. Adjust the pH to between 3 and 4, filter, and dilute the filtrate to 40 ml. with water. For the control (*Solution A*), add 20 mcg. of lead ion (Pb) to 10 ml. of the filtrate, and dilute to 40 ml. For the sample (*Solution B*), dilute the remaining 30 ml. of the filtrate to 40 ml. Add 10 ml. of hydrogen sulfide T.S. to each solution, and allow to stand for 5 minutes. *Solution B* is no darker than *Solution A*.

Lead. Dissolve 1 gram in 20 ml. of diluted hydrochloric acid T.S., evaporate the solution to a volume of about 10 ml. on a steam bath, dilute to about 20 ml. with water, and cool. This solution meets the requirements of the *Lead Limit Test*, page 929, using 5 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

MAGNESIUM PHOSPHATE, TRIBASIC

Trimagnesium Phosphate

$Mg_3(PO_4)_2.xH_2O$ Mol. wt. (anhydrous) 262.86

DESCRIPTION

Tribasic magnesium phosphate may contain 4, 5, or 8 molecules of water of hydration. It occurs as a white, odorless, tasteless crystalline powder. It is readily soluble in dilute mineral acids but is almost insoluble in water.

IDENTIFICATION

A. Dissolve about 200 mg. in 10 ml. of diluted nitric acid T.S. and add, dropwise, ammonium molybdate T.S. A greenish yellow precipitate of ammonium phosphomolybdate forms which is soluble in ammonia T.S.

B. Dissolve 100 mg. in 0.7 ml. of diluted acetic acid T.S. and 20 ml. of water. Add 1 ml. of ferric chloride T.S., let stand for 5 minutes, and filter. The filtrate gives a positive test for *Magnesium*, page 927.

SPECIFICATIONS

Assay. Not less than 98.0 percent and not more than the equivalent of 101.5 percent of $Mg_3(PO_4)_2$, calculated on the ignited basis.

Titration value. Passes test.

Loss on heating. $Mg_3(PO_4)_2.4H_2O$, between 15 and 23 percent; $Mg_3(PO_4)_2.5H_2O$, between 20 and 27 percent; $Mg_3(PO_4)_2.8H_2O$, between 30 and 37 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 10 parts per million (0.001 percent).

Heavy metals (as Pb). Not more than 30 parts per million (0.003 percent).

Lead. Not more than 5 parts per million (0.0005 percent).

TESTS

Assay. Weigh accurately about 200 mg. of the sample, and dis-

solve it in .ture of 25 ml. of water and 10 ml. of diluted nitric acid T.S. Filter, if necessary, wash any precipitate, then dissolve the precipitate by the addition of 1 ml. of diluted nitric acid T.S. Adjust the temperature to about 50°, add 75 ml. of ammonium molybdate T.S., and maintain the temperature at about 50° for 30 minutes, stirring occasionally. Allow to stand for 16 hours or overnight at room temperature. Wash the precipitate once or twice with water by decantation, using from 30 to 40 ml. each time, and pour these two washings through a filter. Transfer the precipitate to the same filter, and wash with potassium nitrate solution (1 in 100) until the last washing is not acid to litmus paper. Transfer the precipitate and filter to the precipitation vessel, add 50.0 ml. of 1 *N* sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein T.S., and then titrate the excess alkali with 1 *N* sulfuric acid. Each ml. of 1 *N* sodium hydroxide is equivalent to 5.714 mg. of $Mg_3(PO_4)_2$.

Titration value. Ignite about 3 grams at about 425° to constant weight, and dissolve, by warming, 2.0 grams of the ignited salt in 50.0 ml. of 1 *N* hydrochloric acid. Cool, add methyl orange T.S., and slowly titrate the excess of 1 *N* hydrochloric acid with 1 *N* sodium hydroxide to a yellow color, shaking the mixture vigorously during the titration. Not less than 29.0 ml. and not more than 30.8 ml. of 1 *N* hydrochloric acid is consumed.

Loss on heating. Weigh accurately about 1 gram and heat at about 425° to constant weight.

Arsenic. A solution of 1 gram in 10 ml. of diluted hydrochloric acid T.S. meets the requirements of the *Arsenic Test*, page 865.

Fluoride. Determine as directed in the *Fluoride Limit Test* under *Magnesium Phosphate, Dibasic*, page 475.

Heavy metals, page 920. Suspend 1.33 grams in 20 ml. of water, and add hydrochloric acid, dropwise, until the sample just dissolves. Adjust the pH to between 3 and 4, filter, and dilute the filtrate to 40 ml. with water. For the control (*Solution A*), add 20 mcg. of lead ion (Pb) to 10 ml. of the filtrate, and dilute to 40 ml. For the sample (*Solution B*), dilute the remaining 30 ml. of the filtrate to 40 ml. Add 10 ml. of hydrogen sulfide T.S. to each solution, and allow to stand for 5 minutes. *Solution B* is no darker than *Solution A*.

Lead. Dissolve 1 gram in 20 ml. of diluted hydrochloric acid T.S., evaporate the solution to a volume of about 10 ml. on a steam bath, dilute to about 20 ml. with water, and cool. This solution meets the requirements of the *Lead Limit Test*, page 929, using 5 mcg. of lead ion (Pb) in the control.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

MAGNESIUM SILICATE

DESCRIPTION

A synthetic form of magnesium silicate in which the molar ratio of magnesium oxide to silicon dioxide is approximately 2:5. It occurs as a very fine, white, odorless, tasteless powder, free from grittiness. It is insoluble in water and in alcohol, but is readily decomposed by mineral acids. The pH of a 1 in 20 slurry is between 6.3 and 9.5.

IDENTIFICATION

A. Mix about 500 mg. with 10 ml. of diluted hydrochloric acid T.S., filter, and neutralize the filtrate to litmus paper with ammonia T.S. The neutralized filtrate responds to the tests for *Magnesium*, page 927.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with a sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a web-like structure.

SPECIFICATIONS

Assay. Not less than 15.0 percent of MgO and not less than 67.0 percent of SiO_2, calculated on the anhydrous basis.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fluoride. Not more than 20 parts per million (0.002 percent).

Free alkali (as NaOH). Not more than 1 percent.

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Soluble salts. Not more than 2 percent.

Water. Not more than 10 percent.

TESTS

Assay for magnesium oxide. Weigh accurately about 1.5 grams, and transfer it into a 250-ml. conical flask. Add 50.0 ml. of 1 *N* sulfuric acid, and digest on a steam bath for 1 hour. Cool to room temperature, add methyl orange T.S., and titrate the excess acid with 1 *N* sodium hydroxide. Each ml. of 1 *N* sulfuric acid is equivalent to 20.15 mg. of MgO.

Assay for silicon dioxide. Transfer about 700 mg., accurately weighed, into a 150-ml. beaker. Add 20 ml. of 1 *N* sulfuric acid, and heat on a steam bath for 1 hour and 30 minutes. Decant the supernatant liquid through an ashless filter paper, and wash the residue, by

decantati ree times with hot water. Treat the residue with 25 ml. of wate and digest on a steam bath for 15 minutes. Finally, transfer the residue to the filter and wash thoroughly with hot water. Transfer the filter paper and its contents to a platinum crucible. Heat to dryness, incinerate, then ignite strongly for 30 minutes, cool, and weigh. Moisten the residue with water, and add 6 ml. of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh. The loss in weight represents the weight of SiO_2.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead. Transfer 10.0 grams of the sample into a 250-ml. flask, and add 50 ml. of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 minutes, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3 filter paper, or equivalent, into a 100-ml. volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml. portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml. of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic. A 10-ml. portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 865.

Fluoride. Weigh accurately 2.5 grams, and proceed as directed in the *Fluoride Limit Test*, page 917.

Free alkali. Add 2 drops of phenolphthalein T.S. to 20 ml. of diluted filtrate prepared in the test for *Soluble salts*, representing 1 gram of magnesium silicate. If a pink color is produced, not more than 2.5 ml. of 0.1 *N* hydrochloric acid is required to discharge it.

Heavy metals. A 5-ml. portion of the *Sample Solution* diluted to 25 ml. with water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

Lead. A 10-ml. portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 929, using 10 mcg. of lead ion (Pb) in the control.

Soluble salts. Boil 10 grams with 150 ml. of water for 15 minutes. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 minutes, and filter until clear. To 75 ml. of the clear filtrate add 25 ml. of water. Evaporate 50 ml. of this solution, representing 2.5 grams of magnesium silicate, in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. The weight of the residue does not exceed 50 mg.

Water. Weigh accurately about 1 gram in a tared platinum crucible provided with a cover. Gradually apply heat to the cr 'e at first, then strongly ignite to constant weight.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Anticaking agent.

MAGNESIUM STEARATE

DESCRIPTION

Magnesium stearate is a compound of magnesium with variable proportions of stearic and palmitic acids. It occurs as a fine, white, bulky, powder, having a faint, characteristic odor. It is unctuous, and is free from grittiness. It is insoluble in water, in alcohol, and in ether. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for salts of fatty acids and fatty acids derived from edible fats sources.

IDENTIFICATION

A. Heat 1 gram with a mixture of 25 ml. of water and 5 ml. of hydrochloric acid. Fatty acids are liberated, floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Magnesium*, page 927.

B. Mix 25 grams of the sample with 200 ml. of hot water, then add 60 ml. of diluted sulfuric acid T.S., and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 minutes. The solidification point of the fatty acids so obtained is not below 54° (see page 931).

SPECIFICATIONS

Assay. Not less than the equivalent of 6.8 percent and not more than the equivalent of 8.0 percent of MgO.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 10 parts per million (0.001 percent).

Loss on drying. Not more than 4 percent.

TESTS

Assay. Boil about 1 gram, accurately weighed, with 50.0 ml. of 0.1

N su[illegible] acid for 10 minutes, or until the fatty acid layer is clear, adding water if necessary to maintain the original volume. Cool, filter, and wash the filter and flask thoroughly with water until the last washing is not acid to litmus. Add methyl orange T.S., and titrate the excess sulfuric acid with 0.1 *N* sodium hydroxide. Each ml. of 0.1 *N* sulfuric acid is equivalent to 2.015 mg. of MgO.

Arsenic. Mix 1 gram of the sample with 10 ml. of hydrochloric acid and 8 drops of bromine T.S., and heat on a steam bath until a transparent layer of melted fatty acid forms. Add 50 ml. of water, boil down to about 25 ml., and filter while hot. Cool, neutralize with a 1 in 2 solution of sodium hydroxide, and dilute to 35 ml. with water. This solution meets the requirements of the *Arsenic Test*, page 865.

Heavy metals, page 920. Place 750 mg. of the sample in a porcelain dish, place 250 mg. of the sample in a second dish for the control, and to each add 5 ml. of a 1 in 4 solution of magnesium nitrate in alcohol. Cover the dishes with 7.6 cm. short stem funnels so that the stems are straight up. Heat for 30 minutes on a hot plate at the low setting, then heat for 30 minutes at the medium setting, and cool. Remove the funnels, add 20 mcg. of lead ion (Pb) to the control, and heat each dish over an Argand burner until most of the carbon is burned off. Cool, add 10 ml. of nitric acid, and transfer the solutions into 250-ml. beakers. Add 5 ml. of 70 percent perchloric acid, evaporate to dryness, then add 2 ml. of hydrochloric acid to the residues, and wash down the inside of the beakers with water. Evaporate carefully to dryness again, swirling near the dry point to avoid spattering. Repeat the hydrochloric acid treatment, then cool, and dissolve the residues in about 10 ml. of water. To each solution add 1 drop of phenolphthalein T.S. and sufficient sodium hydroxide T.S. until the solutions just turn pink, and then add diluted hydrochloric acid T.S. until the solutions become colorless. Add 1 ml. of diluted acetic acid T.S. and a small amount of charcoal to each solution, and filter through Whatman No. 2, or equivalent, filter paper into 50-ml. Nessler tubes. Wash with water, dilute to 40 ml., and add 10 ml. of hydrogen sulfide T.S. to each tube. The color in the solution of the sample does not exceed that produced in the control.

Lead, page 929. Ignite 500 mg. in a silica crucible in a muffle furnace at 475° to 500° for 15 to 20 minutes. Cool, add 3 drops of nitric acid, evaporate over a low flame to dryness, and re-ignite at 475° to 500° for 30 minutes. Dissolve the residue in 1 ml. of a mixture of equal parts by volume of nitric acid and water, and wash into a separator with several successive portions of water. Add 3 ml. of *Ammonium Citrate Solution* and 0.5 ml. of *Hydroxylamine Hydrochloride Solution*, and make alkaline to phenol red T.S. with stronger ammonia T.S. Add 10 ml. of *Potassium Cyanide Solution*. Immediately extract the solution with successive 5-ml. portions of *Dithizone Extraction Solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. [illegible]e the combined extracts for 30 seconds with 20 ml. of dilute [illegible] acid (1 in 100), and discard the chloroform layer. Add to the acid solution exactly 4 ml. of *Ammonia-Cyanide Solution* and 2 drops of *Hydroxylamine Hydrochloride Solution*. Add 10 ml. of *Standard Dithizone Solution*, and shake the mixture for 30 seconds. Filter the chloroform layer through an acid-washed filter paper into a Nessler tube, and compare the color with that of a standard prepared as follows: to 20 ml. of dilute nitric acid (1 in 100), add 5 mcg. of lead ion (Pb), 4 ml. of *Ammonia-Cyanide Solution* and 2 drops of *Hydroxylamine Hydrochloride Solution*, and shake for 30 seconds with 10 ml. of *Standard Dithizone Solution*. Filter through an acid-washed filter paper into a Nessler tube. The color of the sample solution does not exceed that in the control.

Loss on drying, page 931. Dry at 105° to constant weight, using 2-hour increments of heating.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Anticaking agent; binder; emulsifier.

MAGNESIUM SULFATE

Epsom Salt

$MgSO_4.7H_2O$ Mol. wt. 246.47

DESCRIPTION

Small, colorless crystals, usually needle-like, with a cooling, saline, bitter taste. It is freely soluble in water, slowly soluble in glycerin, and sparingly soluble in alcohol. It effloresces in warm, dry air. Its solutions are neutral. A 1 in 20 solution gives positive tests for *Magnesium*, page 927, and for *Sulfate*, page 928.

SPECIFICATIONS

Assay. Not less than 99.5 percent of $MgSO_4$ after ignition.

Loss on ignition. Between 40 and 52 percent.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 10 parts per million (0.001 percent).

Selenium. Not more than 30 parts per million (0.003 percent).

TESTS

Assay. Weigh accurately about 500 mg. of the residue obtained i[illegible] the test for *Loss on ignition*, dissolve it in a mixture of 50 ml. of wate[illegible]

and 1 ml. of hydrochloric acid, dilute to 100.0 ml. with water, and mix. Transfer 50.0 ml. of this solution into a 250-ml. Erlenmeyer flask, add 10 ml. of ammonia-ammonium chloride buffer T.S. and 12 drops of eriochrome black T.S., and titrate with 0.1 *M* disodium ethylenediaminetetraacetate until the wine-red color changes to pure blue. Each ml. of 0.1 *M* disodium ethylenediaminetetraacetate is equivalent to 12.04 mg. of $MgSO_4$.

Loss on ignition. Weigh accurately about 1 gram in a crucible, heat at 105° for 2 hours, then ignite in a muffle furnace at 450° ± 25° to constant weight.

Arsenic. A solution of 1 gram in 10 ml. of water meets the requirements of the *Arsenic Test*, page 865.

Heavy metals. A solution of 2 grams in 25 ml. of water meets the requirements of the *Heavy Metals Test*, page 920, using 20 mcg. of lead ion (Pb) in the control (*Solution A*).

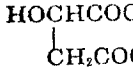
Selenium. A solution of 2 grams in 40 ml. of dilute hydrochloric acid (1 in 2) meets the requirements of the *Selenium Limit Test*, page 953.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Nutrient; dietary supplement.

MALIC ACID

DL-Malic Acid; Hydroxysuccinic Acid



$C_4H_6O_5$ Mol. wt. 134.09

DESCRIPTION

White or nearly white, crystalline powder or granules having a strongly acid taste. One gram dissolves in 0.8 ml. of water and in 1.4 ml. of alcohol. Its solutions are optically inactive.

SPECIFICATIONS

Assay. Not less than 99.5 percent of $C_4H_6O_5$.

Melting range. Between 130° and 132°.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Fumaric acid. Not more than 0.5 percent.

Heavy metals (as Pb). Not more than 20 parts per million (0.002 percent).

Lead. Not more than 10 parts per million (0.001 percent).

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SPECTROPHOTOMETRIC DETERMINATION OF SERUM MAGNESIUM BY ERIOCHROME BLACK T

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Clinical studies of the changes in the magnesium (Mg) concentration of body fluids in disease have been impeded by the technical difficulties involved in the assay. In the original (early) methods of Kramer and Tisdall,[10] of Briggs,[2] and of Denis,[4] the calcium is removed from 2 ml. of serum as the insoluble oxalate; the magnesium is then precipitated as magnesium ammonium phosphate and the latter is estimated by a colorimetric phosphate determination. Other less cumbersome methods for the determination assay of magnesium involve the use of 8-hydroxyquinoline, either as a precipitant for preliminary isolation[9] or as a reagent for inducing fluorescence in a fluorometric procedure assay.[12, 13] Titan yellow, which under alkaline conditions forms a red-colored lake with magnesium, has gained considerable popularity as a somewhat simplified procedure.[1, 17] Titrimetric methods based upon the use of ethylenediaminetetraacetic acid (EDTA) as titrant and Eriochrome black T (EBT) (Geigy Pharmaceuticals, Yonkers, N. Y.) as indicator have also been widely used.[7] The advent of atomic absorption spectrophotometry has greatly simplified the technical procedure at the expense of an instrument costing several thousand dollars.

The method described herein is based upon the use of the alkaline earth metal indicator Eriochrome black T which forms a red chromophore with magnesium.[3, 5, 14, 15] The response of EBT to calcium is completely suppressed by incorporating a small quantity of the barium salt of ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) in the color reagent.[6] EBT was first used in a colorimetric procedure for the determination of magnesium in water by Young and associates,[18, 19] and was first adapted to biologic material by Smith[16] and Levine and Cummings.[11] In their procedures, the calcium is removed (precipitated) from 2 ml. of blood serum as the oxalate. Protein is then precipitated from an aliquot of the calcium-free serum. An aliquot of the protein-free supernatant fluid is then added to the EBT color reagent at pH 11.5 and the absorbance is measured at 530 mμ.

In our method removal of calcium is unnecessary; the technician simply adds 0.2 ml. of 1:1 protein-free extract of serum to 5 ml. of the EBT color reagent and compares the absorbance at 530 mμ with that of a magnesium standard.

Received September 18, 1967.

MATERIALS AND METHODS

Reagents

1. *0.75 N Perchloric acid ($HClO_4$).* Add either 65 ml. of A. C. S. (reagent grade), 70% w/w, or 84 ml. of 60% w/w $HClO_4$ to sufficient deionized water to make 1 l. Adjust by titration.

2. *1.0 M Sodium perchlorate ($NaClO_4$).* Dissolve 70 Gm. of $NaClO_4$-H_2O* in sufficient deionized water to make 500 ml.

3. *Protein-precipitating reagent ($HClO_4$-$NaClO_4$) (PPR).* Mix equal volumes of 0.75 N perchloric acid and 1.0 M sodium perchlorate. This reagent may also be prepared by mixing equal volumes of 1.75 N perchloric acid and 1.0 N sodium hydroxide. The sedimentation ability of the precipitated protein during centrifugation is improved by incorporating 0.1 ml. of Brij 30 (polyoxyethylene (4) lauryl ether)† per 500 ml. of PPR.

4. *Eriochrome black T solution, 1.0 mg. per ml.* Dissolve 100 mg. of EBT‡ (1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid sodium salt) in 100 ml. of *N*-methyl-2-pyrolidone.§ Store in refrigerator.

* Available from G. Frederick Smith Chemical Company, Columbus, Ohio (Item 92).

† Atlas Chemical Industries, Inc., Wilmington, Del. 19899.

‡ G. Frederick Smith Chemical Company (Item 246).

§ General Aniline & Film Corporation, Dyestuff & Chemical Division, New York, N. Y.

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5. *Barium EGTA solution.* Dissolve 3.8 Gm. (10 mM) of ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA)* in 60 ml. of water and 6 ml. of 5 N NaOH. Then add 2.6 Gm. of barium chloride (10.64 mM) previously dissolved in 25 ml. of water. Adjust the final volume to 100 ml. with water. This reagent contains a 6.4% w/v excess of barium by design.

6. *10 M Aminoethanol buffer, pH 11.65 ± 0.1.* Into a 100-ml. glass-stoppered graduated cylinder, place 60 ml. of 2-aminoethanol (No. 1597 Eastman Organic Chemicals or Baker analyzed reagent No. 9314 (J. T. Baker Chemical Company, Phillipsburg, N. J.)), 35 ml. of deionized water, and 5 ml. of 50% v/v hydrochloric acid. Add the acid slowly, mixing after each addition.

7. *EBT color reagent for magnesium, pH 11.0 ± 0.1.* Into a 100-ml. glass-stoppered graduated cylinder, place 5 ml. of 10 M aminoethanol buffer. Add 5 ml. of EBT solution and 1 ml. of barium-EGTA solution. Dilute to the 100-ml. mark with deionized or double distilled water. The pH of this reagent is approximately 11.0. This reagent keeps for only 4 hr.

8. *Stock standard magnesium solution, 1 mg. per ml.* Dissolve 1.0 Gm. of magnesium metal turnings, analytical reagent, in the minimal quantity of 4 N hydrochloric acid (approximately 20 ml.) and dilute to 1 l. with deionized water.

9. *Working standard magnesium solutions.* A working standard equivalent to 2 mg. per 100 ml. under test conditions is prepared by adding 1 ml. of stock standard magnesium solution (1 mg. per ml.) to a 100-ml. volumetric flask. $HClO_4$-$NaClO_4$ protein-precipitating reagent, 50 ml., is then added and the vessel is filled to the 100-ml. mark with deionized water. Working standards equivalent to 1 mg. per 100 ml. and 4 mg. per 100 ml. under test conditions are similarly prepared by using 0.5 ml. of stock standard and 2 ml. of stock standard, respectively.

10. *Reagent blank solution.* Mix equal volumes of $HClO_4$-$NaClO_4$ PPR and deionized water.

* Also designated ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid. Available from Eastman Organic Chemicals Company, Rochester, N. Y. (Cat. No. 8276).

Procedure

Add 1 ml. of serum to 1 ml. of perchloric acid-sodium perchlorate protein-precipitating reagent. Mix and let stand for 30 min. Centrifuge. Add 0.2 ml. of the clear supernatant to 5 ml. of EBT color reagent No. 7 and measure the absorbance at 530 mμ *versus* the blank. The standard and reagent blank are prepared by adding 0.2 ml. of the appropriate solution to 5 ml. of the EBT color reagent. A set of standards must be run with each new group of test samples.

Calculations

$$\frac{U}{S} \times 2 = \text{mg. of magnesium per 100 ml. of blood serum}$$

$$\frac{U}{S} \times 1.64 = \text{mEq. of magnesium per l. of serum}$$

where U is the absorbance of the test sample and S is the absorbance of the 2 mg. per 100 ml. (equivalent concentration) standard. A high quality spectrophotometer such as the Beckman Model B, DB, DU (Beckman Instruments, Inc., Fullerton, Calif.) or the Gilford Model 300 (Gilford Instrument Laboratories, Oberlin, Ohio) should be used to measure the absorbance.

DISCUSSION

EBT, 1-(1-Hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid sodium salt, forms metallochromic complexes with several different cations, including magnesium, calcium, zinc, copper, and iron. The absorptivity of the EBT metal complexes of calcium, magnesium, barium, copper, iron, strontium, and zinc at 530 mμ using our EBT color reagent is depicted in Table 1. The response of the reagent to calcium is completely suppressed by incorporating a small amount of the barium salt of EGTA in the reagent.[6]

The displacement reaction, $Mg^{++} + Ca^{++} + Ba\text{-EGTA} \rightarrow Mg^{++} + Ca\text{-EGTA} + Ba^{++}$, complexes the calcium and releases an equivalent quantity of barium with which the EBT does not react under test conditions. The reaction proceeds because the stability of the EGTA complex of barium is inter-

TABLE 1

ABSORPTIVITY OF EBT-METAL COMPLEXES AT 530 mμ DEVELOPED IN EBT COLOR REAGENT NO. 7

Metal	Concentration	Absorbance at 530 mμ	$E^{1\%}_{1cm}$ *	Molar Absorptivity†
	μg./ml.			
Mg	2	1.337	6,685	16,000
Ca‡	2	0.575	2,875	11,500
Ba‡	2	0.000		
Cu	2	0.078	390	2,457
Fe	2	0.029	14.5	81.2
Zn	2	0.471	2,355	15,307
Sr	2	0.000		

* Standard symbol for absorptivity (extinction coefficient) which is the absorbance of a 1% (w/v) solution for a 1-cm. light path.

† Molar absorptivity is the absorbance of a 1 M solution for a 1-cm. light path.

‡ Barium EGTA was omitted from the EBT color reagent.

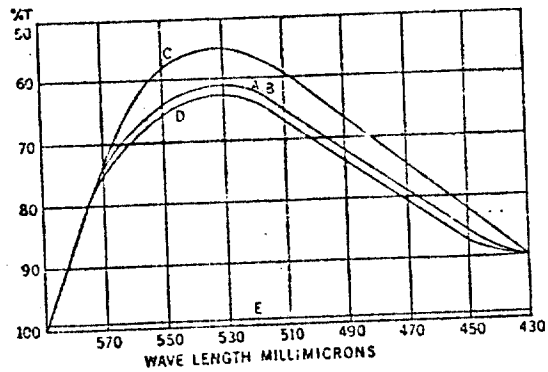

FIG. 1. Spectral transmittance curves at pH 11.0 for Eriochrome black T reagent No. 7 containing *A*, 5.0×10^{-6} M magnesium, *B*, 5.0×10^{-6} M magnesium and 2.5×10^{-5} M calcium (equivalent to 2 mg. per 100 ml. of serum Mg and 10 mg. per 100 ml. of serum Ca under test conditions; the curves coincide), and *E*, 5.0×10^{-5} M calcium measured against the blank reagent solution, containing neither calcium nor magnesium. *Curves C* and *D* were made with modified EBT reagent containing no barium-EGTA, but containing *C*, 2.5×10^{-5} M calcium, and *D*, 5.0×10^{-6} M magnesium.

mediate to that of magnesium and of calcium, the stability of the calcium complex being the greatest. Consequently calcium, but not magnesium, will displace barium from its EGTA complex. If deemed necessary, zinc and copper and iron can be complexed and rendered completely unreactive by incorporating a trace of cyanide ion in the reagent.

Figure 1 shows spectral transmittance curves at pH 11.0 for EBT reagent No. 7 and for modified EBT reagent containing no barium-EGTA. It can be seen that, in the absence of barium-EGTA, the response of the reagent to a calcium content of 2.5×10^{-5} M (equivalent to a serum calcium concentration of 10 mg. per 100 ml. under test conditions) (*Curve C*) exceeds that of a reagent magnesium concentration of 5×10^{-6} M (equivalent to a serum magnesium concentration of 2 mg. per 100 ml. under test conditions) (*Curve D*) while EBT color reagent No. 7 containing barium-EGTA does not respond at all to a calcium concentration of 5×10^{-5} M (equivalent to a serum calcium concentration of 20 mg. per 100 ml. under test conditions) (*Curve E*). Also, the response of the EBT reagent No. 7 to a magnesium concentration of 5.0×10^{-6} M (*Curve A*) and to a combined magnesium concentration of 5.0×10^{-6} M and a calcium concentration of 2.5×10^{-5} M (*Curve B*) is identical since both curves coincide.

Figure 2 shows the relation between the pH of the EBT reagent and the absorbance of a fixed concentration of magnesium (1.4×10^{-5} M) contained in the reagent, measured at 530 mμ. The greatest sensitivity of the reagent for magnesium is achieved at pH 11.0. Figure 3 shows the relation of absorbance to total magnesium concentration at 530 mμ for EBT color reagent No. 7

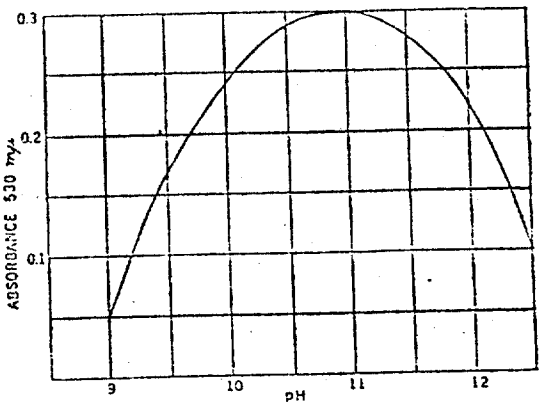

FIG. 2. Plot of the relation between the pH of the EBT color reagent and the absorbance of a fixed concentration of magnesium (1.4×10^{-5} M) contained in the reagent, measured at 530 mμ against the reagent blank.

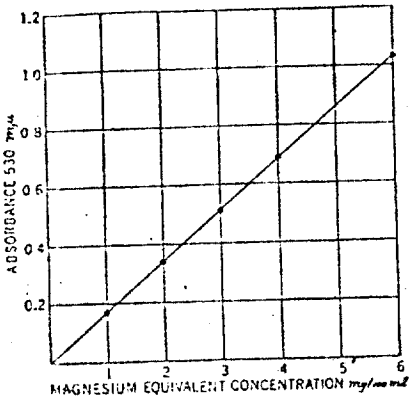

FIG. 3. Absorbance *versus* equivalent concentration of magnesium, mg. per 100 ml., for EBT color reagent No. 7 measured under test conditions at 530 mμ against the reagent containing no magnesium. An equivalent serum concentration of 2 mg. per 100 ml. represents an actual concentration of 0.3846 μg. of magnesium per ml. of color reagent.

containing 0 to 120 μg. per 100 ml. of magnesium, representing 0 to 6 mg. of magnesium per 100 ml. of blood serum under test conditions. The concentration curve is linear and passes through the origin. The absorbance of both serum-reagent and of aqueous magnesium-reagent solution developed under test conditions shows no significant change for 60 min. The presence of the carbonate ion does not interfere. Phosphate ion equivalent to 30 mg. per 100 ml. of phosphorus added to a serum does not cause any magnesium absorbance change when compared with the same untreated serum.

Table 2 is a summary of the results of 26 serum magnesium analyses performed in duplicate both by the EBT procedure described herein and by atomic absorption spectrophotometry. A Perkin-Elmer model 303 instrument (Perkin-Elmer Corporation, Norwalk, Conn.) was used. The serum samples were prepared for this analysis by being diluted 1:50 with a 0.5% w/v lanthanum solution* before being aspirated into the flame. The atomic absorption analyses were performed by Dr. Edwin M. Richardson, biochemist at the Delaware Division of the Wilmington Medical Center, inasmuch as the authors do not possess such an instrument.

*12.7 Gm. of $La\ Cl_3 \cdot 6H_2O$ + 16 ml. of concentrated HCl + 60 ml. of butanol + 0.2 ml. of octanol + H_2O qs. ad. 1000 ml.

TABLE 2

COMPARISONS OF SERUM MAGNESIUM ANALYSES

Serum No.	Magnesium		Difference
	Atomic absorption*	EBT procedure	
	mg./100 ml.		
1	1.9	1.85	+0.05
2	2.18	2.15	+0.03
3	2.54	2.55	−0.01
4	2.12	2.00	0.12
5	2.00	2.00	0.00
6	1.78	1.70	0.08
7	2.06	2.10	−0.04
8	2.04	2.05	−0.01
9	2.00	2.15	−0.15
10	2.18	2.35	−0.17
11	2.08	2.05	0.03
12	2.34	2.50	−0.16
13	1.81	1.85	−0.04
14	5.00	5.20	−0.20
15	3.80	3.90	−0.10
16	2.40	2.40	0.00
17	2.39	2.35	0.04
18	2.45	2.50	−0.05
19	2.91	2.90	0.01
20	2.41	2.35	0.06
21	3.03	3.00	0.03
22	2.36	2.45	−0.09
23	2.00	2.10	−0.10
24	3.01	3.20	−0.19
25	2.93	2.95	−0.02
26	2.44	2.40	0.04
Mean	2.467 ± 0.69	2.50 ± 0.73	−0.0323

* Perkin-Elmer Model 303 atomic absorption spectrophotometer was used. The sample was diluted with lanthanum chloride solution.

The mean of the values obtained by atomic absorption is 2.467 mg. per 100 ml. of serum with a standard deviation of 0.69 mg. per 100 ml., whereas the mean of the values obtained by the authors' EBT procedure is 2.50 mg. per 100 ml. with a standard deviation of 0.73 mg. per 100 ml.

The results in Table 2 were also analyzed statistically by means of the *t* test.[8] The mean difference between methods was d = 0.0323 mg. per 100 ml. and SD, the standard deviation of the differences, was 0.088. Consequently,

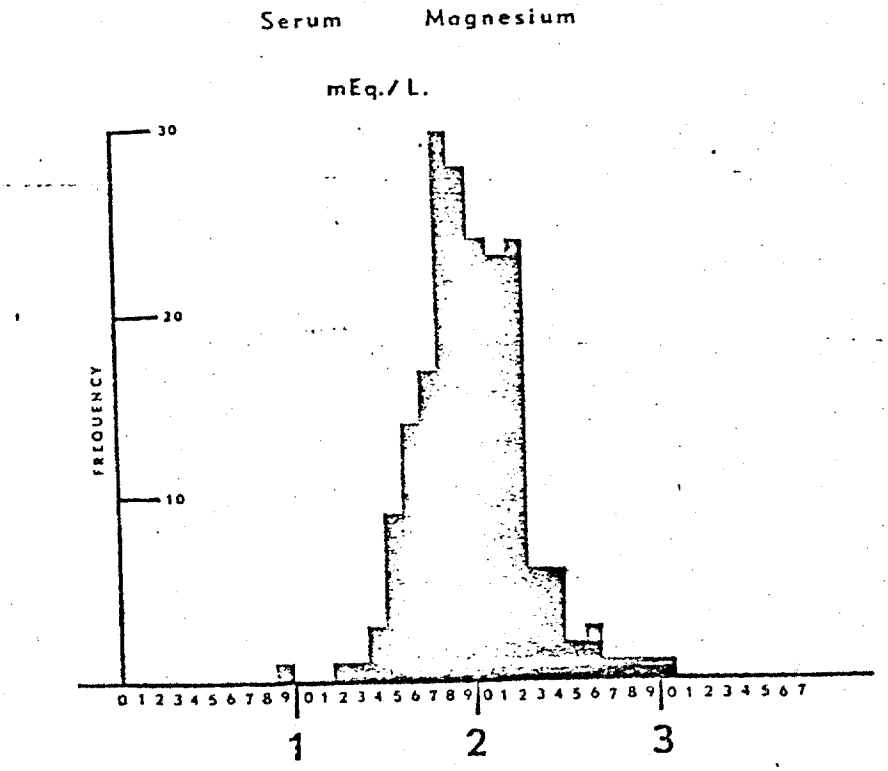

FIG. 4. Histogram depicting the range of values obtained by analyzing the sera of 200 random hospital patients for magnesium by means of the authors' EBT procedure. Each assay was performed in duplicate.

$$t = \frac{d}{\overline{SD}}\sqrt{N} = \frac{0.0323}{0.0SS}\sqrt{26} = 1.835$$

Inasmuch as t_{25} (95% limits) = 2.06, it is concluded that the data give no evidence of a systematic difference between methods. Analysis of a serum pool for magnesium by means of the EBT procedure on 15 different days gave a mean value of 2.24 mg. per 100 ml. with a standard deviation of 0.0977 mg. per 100 ml. and a coefficient of variation of 4.36%.

Figure 4 shows a histogram depicting the results of duplicate analyses of 200 sera for magnesium selected at random from the daily laboratory workload and analyzed by means of the EBT procedure described here. The range is from 0.9 to 3.0 mEq. per l. (1.08 to 3.6 mg. per 100 ml.). Statistical analysis of these values gives a mean serum magnesium value of 1.94 mEq. per l. (2.33 mg. per 100 ml.) with a standard deviation of 0.2126 mEq. per l. Because the magnesium values represented by the histogram were derived from ill hospital patients, the mean does not indicate the mean normal value established by this method. However, since 89% of the values (179 of 200) occur over the interval from 1.5 mEq. per l. to 2.3 mEq. per l. (1.8 to 2.76 mg. per 100 ml.), which corresponds to the range given for normal values by several different authors,[7] it may be inferred that these also represent the approximate normal range for the EBT method.

SUMMARY

A manual spectrophotometric method for the determination of serum magnesium using Eriochrome black T (EBT) is described. A protein-free filtrate of serum is conveniently prepared by mixing and centrifuging 1 ml. of serum with 1 ml. of specially prepared perchloric acid-sodium perchlorate precipitating reagent (0.375 M perchloric acid, 0.5 M sodium perchlorate). Then 0.2 ml. of the protein-free filtrate is mixed with 5 ml. of alkaline-buffered Eriochrome black T color

reagent containing an optimal amount of the barium salt of ethylenebis(oxyethylenenitrilo)tetraacetic acid which completely suppresses the response of the EBT to calcium. The absorbance of the magnesium-EBT chromaphore is measured at 530 mμ *versus* the reagent blank. Excellent correlation was obtained by comparing results of the EBT method of analysis with those obtained by analyzing the same sera for magnesium by means of atomic absorption spectrophotometry.

Acknowledgment. Dr. Edwin M. Richardson, biochemist at the Delaware Division of the Wilmington Medical Center, Wilmington, Del., checked by means of atomic absorption the serum magnesium values obtained with our EBT procedures.

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21(1): 394-398, 1951

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THE SPECTROPHOTOMETRIC DETERMINATION OF MAGNESIUM IN HUMAN SERUM*

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The estimation of small quantities of magnesium by the use of titan (thiazole) yellow was developed by Kolthoff.[5] Subsequent modifications of the original procedure have been concerned principally with stabilization of the red lake formed by magnesium and titan yellow in the presence of alkali. Various protective colloids, including gelatin,[1] gum ghatti,[2] polyvinyl alcohol,[4] and starch,[7] have been employed in an attempt to produce uniform dispersion of the red lake, and hydroxylamine has been used in an effort to prevent fading of the color.[3, 6] Preliminary removal of protein has been accomplished either by trichloracetic acid[3] or by tungstic acid.[6]

METHOD

The method of sample preparation appears fully as important as the selection of the proper protective colloid. The procedure presented in this manuscript requires only 1 ml. of plasma or serum and consists of the following steps:

1. Destruction of plasma sample in a 50-ml. Erlenmeyer flask by 5 ml. of fuming nitric acid, followed by evaporation to dryness, and solution of the residue by the addition of 2 drops of concentrated nitric acid and 3 ml. of distilled water.
2. Consecutive addition of 1.0 ml. of polyvinyl alcohol-sodium lauryl sulfate reagent, 2.0 ml. of titan yellow solution, and 3.0 ml. of 2 N sodium hydroxide, with mechanical stirring as the alkali is introduced.
3. Colorimetric comparison at 550 mμ in a Beckman Spectrophotometer against standards containing known amounts of magnesium and the other inorganic constituents of plasma, and subjected to the same preparation as the samples.

Reagents. 1. Polyvinyl alcohol-sodium lauryl sulfate reagent.

To 250 ml. of distilled water add 0.125 grams of sodium lauryl sulfate (Dreft). Mix well and add 2.5 grams of polyvinyl alcohol (Dupont Elvanol Code 70-05, 98 to 100 per cent hydrolyzed, low viscosity). Heat to 60 C. to effect a clear solution.

2. Titan yellow solution 0.05 per cent.
3. 2 N sodium hydroxide C. P.
4. Urea C. P. 36 grams per liter.
5. Salt solution; C. P. chemicals.

8.37 grams of NaCl, 0.34 grams KCl, 0.25 grams $CaCO_3$, 0.55 grams diammonium hydrogen phosphate, 0.17 grams ammonium sulfate, 0.004 grams $CuSO_4 \cdot 5\ H_2O$ and 5 ml. of concentrated hydrochloric acid are made up to 1 liter.

* Supported in part by grants-in-aid from the National Heart Association and the Michigan Heart Association.

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#3997

6. Magnesium standard solution.

Dissolve 20.3 milligrams of C. P. magnesium metal in 3 ml. of 1:1 hydrochloric acid. Dilute to 1 liter. This solution contains 1.67 mEq. of magnesium per liter.

Instrumentation. Beckman Model DU Quartz Spectrophotometer; Corex Cells; tungsten source; 550 mμ, blue photocell, slit 0.4 mm.

Preparation of sample. To 1.0 ml. of plasma or serum in a 50-ml. Erlenmeyer flask add 5 ml. of fuming nitric acid. Evaporate to dryness on a medium hot plate. Add 4 or 5 drops of concentrated nitric acid to the hot dried residue to insure complete destruction of protein. Remove from the hot plate and wash down the sides

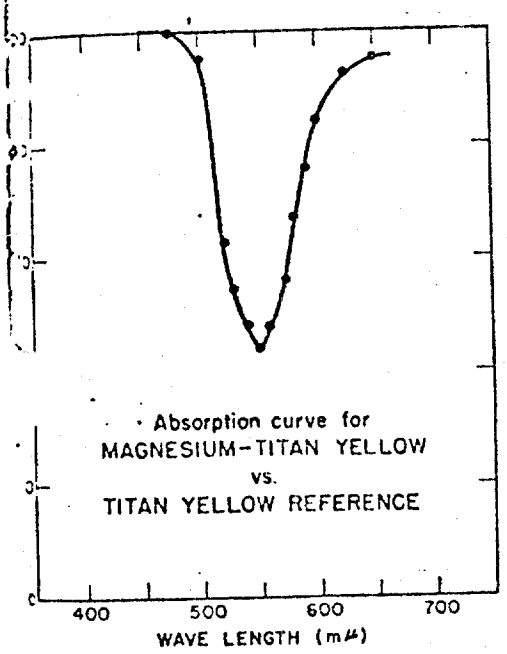

FIG. 1

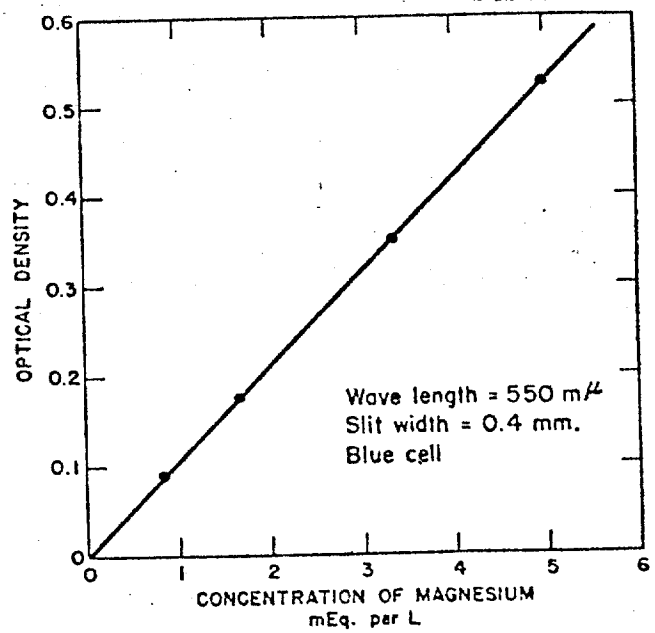

FIG. 2

FIG. 1. Absorption curve for magnesium-titan yellow vs. titan yellow reference

FIG. 2. Standardization curve showing linear relationship of optical density and concentration of magnesium.

of the flask with 5 ml. of distilled water. Return to the hot plate and evaporate until all fumes are dispelled. After cooling, add, in order, 2 drops of concentrated nitric acid, 3.0 ml. of distilled water, 1.0 ml. of polyvinyl alcohol-sodium lauryl sulfate reagent, 2.0 ml. of titan yellow solution, and 3.0 ml. of 2 N sodium hydroxide. As the alkali is introduced, stir mechanically to insure homogeneity of mixing during the formation of the complex. After 15 minutes' standing, make a color comparison against a blank containing water, polyvinyl alcohol reagent, titan yellow solution and 2 N sodium hydroxide in the same amounts as used in the sample preparation.

Preparation of standards. To 1.0 ml. of salt solution in each of four 50-ml. Erlenmeyer flasks, add 0.5, 1.0, 2.0 and 3.0 ml., respectively, of magnesium standard solution. To each flask add 1.0 ml. of the urea solution and 5.0 ml. of fuming nitric acid. Digest on a medium hot plate, taking the samples to dryness.

Remove from the hot plate and wash down the sides of the flask with 5 ml. of distilled water. Return to the hot plate and evaporate until all fumes are dispelled. From this point onward, the procedure is identical with that described under sample preparation.

Results. The absorption peak for the titan yellow-magnesium standard in reference to the titan yellow blank in alkaline media is illustrated in Figure 1. Peak

TABLE 1

RECOVERIES OF MAGNESIUM BY SPECTROPHOTOMETRIC METHOD

	mEq. MG. PRESENT*	mEq. MG. ADDED	mEq. MG. FOUND	ERROR mEq.	PER CENT ERROR
Pooled plasma I	1.33	1.00	2.29	−0.04	1.7
	1.33	1.67	3.00	−0.01	0.3
	1.33	3.34	4.79	+0.11	2.3
	1.33	0.84	2.17	0.00	0
	1.33	1.67	3.08	+0.08	2.6
	1.33	3.34	4.37	−0.30	6.9
	1.33	0.84	2.20	+0.03	2.3
	1.33	1.67	2.62	−0.38	12.7
	1.33	3.34	4.66	−0.01	0.2
Pooled serum II	1.72	0.84	2.46	−0.10	3.9
	1.72	1.67	3.50	+0.11	3.2
	1.72	3.34	5.00	−0.06	1.2
Pooled serum, ½ strength	0.94	0.84	1.76	−0.02	1.1
	0.94	1.00	1.91	−0.03	1.6
	0.94	1.00	2.02	+0.08	4.1
	0.94	1.67	2.48	−0.13	5.0

* Average of 5 independent determinations, using the titan yellow procedure.

TABLE 2

COMPARISON OF RESULTS OF EMISSION SPECTROGRAPHIC AND SPECTROPHOTOMETRIC (TITAN YELLOW) METHODS FOR QUANTITATIVE ESTIMATION OF MAGNESIUM

SERUM	EMISSION SPECTROGRAPH mEq. MG.	TITAN YELLOW METHOD mEq. MG.
A	1.87	1.84
B	1.79	1.95
C	1.56	1.48
Pooled plasma III, (half strength)	0.92	0.94

absorption for the magnesium complex occurs at 550 mμ in the presence of the polyvinyl alcohol-sodium lauryl sulfate stabilizer. Therefore, 550 mμ was the optimal wave length for quantitation of magnesium by this procedure. The use of 520 mμ,[2] 530 mμ[3] and 540 mμ[6] by other workers may be accounted for by differences in the stabilizer. The calibration curve (Figure 2) is included merely to show the linear nature of the quantitation of magnesium by this method and the conformity to Beer's Law.

Table 1 lists recoveries obtained on the addition of a known amount of magnesium to 1.0-ml. samplse of a pooled plasma and serum and one diluted pooled serum. Repeated analysis of these three specimens yielded 1.33, 1.72 and 0.94 mEq. magnesium per liter, respectively. It will be noted that with the exception of one determination, the recoveries are quite acceptable.

Table 2 shows a comparison between analytical results for magnesium by the emission spectrograph and by the titan yellow colorimetric procedure.

DISCUSSION

Preparation of standard. Standards prepared in accordance with the procedure described above yielded linear calibration curves that were consistently reproducible. Hence, the same calibration curve may be used indefinitely, provided that strict adherence to all details of the procedure for sample analysis is maintained.

The measurement of magnesium in the presence of similar anions in both the standard and sample tends to lend uniformity to the procedure. Calibration curves differed when the procedure was altered, either by leaving out the salt solution or by failing to carry the standard through nitric acid digestion.

The addition of urea to the standard appeared to serve two purposes: (1) promotion of uniform boiling through its decomposition in CO_2 and ammonia, and (2) improvement in the reproducibility of the calibration curves in the lower range of concentrations.

Preparation of sample. The destruction of protein by nitric acid oxidation employed in this procedure avoids possible losses of magnesium in the course of protein precipitation and filtration utilized by previous workers. Furthermore, this procedure circumvents errors which may come from the influence of excesses of protein precipitating agents, such as tungstic acid, on the stability of the titan yellow-magnesium complex.

Stabilization of the color complex. The addition of polyvinyl alcohol alone to the digested and redissolved sample did not give a completely transparent solution. The inclusion of sodium lauryl sulfate in the polyvinyl alcohol reagent not only yielded a transparent solution of the sample, but also stabilized the titan yellow-magnesium complex. The red color of the titan yellow-magnesium complex reached its maximal intensity within 15 minutes and remained stable for at least 12 hours. The sodium lauryl sulfate-polyvinyl alcohol reagent appeared to be superior to other protective colloids from the standpoint of color decay.

SUMMARY

A rapid and accurate modification of the titan yellow method for the determination of magnesium in 1-ml. volumes of plasma or serum is described. The innovations of this method include destruction of protein by nitric acid digestion, the use of a sodium lauryl sulfate-polyvinyl alcohol reagent as stabilizer, the selection of a 550-mμ wave length for quantitation, and the preparation of calibration curves from standards containing urea and the electrolytes normally present in plasma.

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finding that, on electrophoresis, 90% of the supernatant enzyme migrates to the anode, whereas the anode component of the mitochondrial enzyme represents only 14% of the total (Wieland, Pfleiderer, Haupt & Worner, 1959). Evidence for two components with malic-dehydrogenase activity was obtained during chromatography of preparations from cauliflowers and peas (Figs. 1 and 2), but the separation did not appear to be connected with differences in specificity (Table 2).

It appears to the author that the existence of protein interactions and the likelihood of proteolytic activity modifying the structure of enzymes without destroying catalytic activity make it quite likely that electrophoresis or chromatography will indicate the presence of two peaks of activity in many enzyme preparations. The observation of multiple peaks may be unimportant unless a physiological difference between the molecular species of the enzyme is found. The existence of different enzymes catalysing the same reaction is potentially of great interest. The discovery of two examples in bacteria, the existence of two threonine deaminases, one of which is biosynthetic whereas the other is adeptive and degradative (Umbarger & Brown, 1957), and the demonstration of two distinct systems for the synthesis of acetolactate, only one of which appears to participate in valine biosynthesis (Halpern & Umbarger, 1959), suggest that the pattern of two enzymes catalysing the same reaction may be found in other biological systems.

SUMMARY

1. A method of purifying malic dehydrogenase from pea epicotyls is described. A 50-fold purification was obtained by ammonium sulphate fractionation, treatment with calcium phosphate gel and chromatography on diethylaminoethylcellulose.

2. Evidence is presented for the view that plant malic dehydrogenase, like the corresponding animal enzyme, is an α-hydroxydicarboxylic acid dehydrogenase.

3. A number of kinetic constants have been evaluated.

4. Slight differences between malic dehydrogenase prepared from mitochondria and from the supernatant remaining after removing mitochondria from a homogenate were noted. Two peaks with malic-dehydrogenase activity were observed during chromatography but all preparations appeared to have the same substrate specificity.

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The Determination of Magnesium in Biological Materials by Atomic Absorption Spectrophotometry

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The wide variety of methods which have been employed for the determination of magnesium in biological materials serves to illustrate the fact that no method is completely satisfactory. The majority of methods are chemical and the earlier procedures require an initial separation of magnesium, either as the insoluble ammonium phosphate salt (Briggs, 1922; Denis, 1922), or as the complex with 8-hydroxyquinoline (Greenberg & Mackey, 1932). Direct combination with the dye

* Medical Research Council External Staff.

Titan yellow has been widely used (Gillam, 1941; Garner, 1946; Orange & Rhein, 1951), whereas more recently methods utilizing other dyestuffs and titration with ethylenediaminetetra-acetic acid have been developed (Smith, 1955; Wilkinson, 1957). Unfortunately many of these methods are inaccurate or of very limited application, owing to interference by other constituents of biological materials.

It is desirable that any method should be applicable to the analysis of ashed specimens of faeces and food or other cellular material in addition to biological fluids such as serum and urine. Modifications of existing ammonium phosphate-separation methods have been shown to yield satisfactory results with all types of specimen (Heaton, 1960), but as the analysis requires 2 days a more rapid method is necessary for many purposes.

During the past few years flame photometry has been applied to the problem (Vallee & Margoshes, 1956; MacIntyre & Davidsson, 1958), but the method is of low sensitivity, owing to the weak emission of the magnesium ion compared with the flame background, unless either very high flame temperatures or integrating systems are employed (Exley & Sproat, 1958). Greater sensitivity may be obtained by utilizing the principle of atomic absorption spectroscopy suggested by Walsh (1955). This principle has been used by Allan (1958) and David (1958) to determine several metals in biological materials after preliminary ashing, and by Willis (1960*a*, *b*) to estimate calcium and magnesium in serum after simple dilution.

The estimation of magnesium by atomic absorption spectroscopy involves passing light containing a high proportion of magnesium resonance radiation through a flame. Into the flame are aspirated solutions whose magnesium content is to be determined, and the light absorbed at 285.213 mμ is compared with the absorption of a standard magnesium solution treated similarly. An instrument suitable for atomic absorption spectrophotometry has been constructed in this laboratory and the method examined for the analysis of both biological fluids and solutions of the ash obtained from cellular materials.

The classical magnesium ammonium phosphate separation method has been critically examined and its accuracy determined. By comparing the results of this method with those obtained from the same specimens analysed by atomic absorption spectrophotometry, the absolute accuracy of the latter method has been estimated.

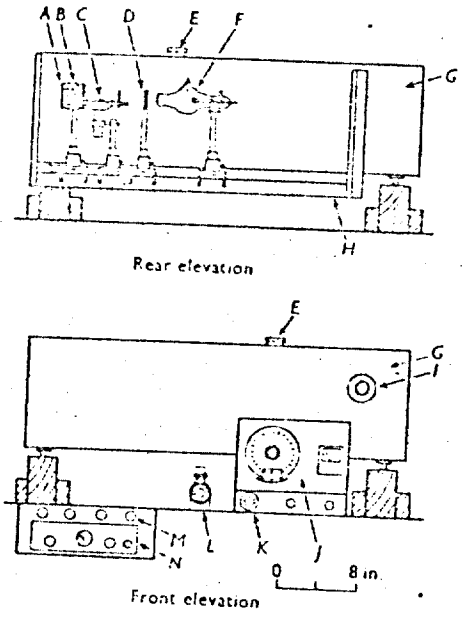

Fig. 1. Arrangement of atomic absorption spectrophotometer. *A*, Entrance slit of monochromator; *B*, mirror; *C*, burner; *D*, condenser lens; *E*, wavelength control; *F*, magnesium lamp; *G*, monochromator and detection system; *H*, optical bench; *I*, slit-width control; *J*, extinction scale and null-point meter; *K*, needle valve; *L*, atomizer and spray chamber; *M*, sensitivity controls; *N*, lamp-current controls.

EXPERIMENTAL

Description of the atomic absorption spectrophotometer

The arrangement of the principal components (the magnesium lamp, atomizer, spray chamber, burner, monochromator and detection system) is shown in Fig. 1.

Magnesium lamp. The lamp, generating light of the magnesium resonance frequency, is a quartz-windowed,

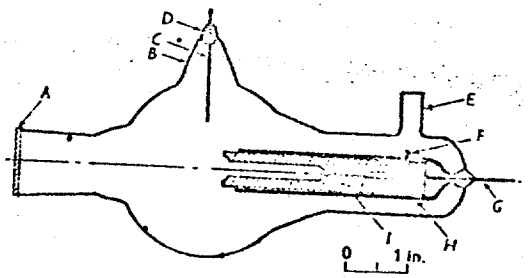

Fig. 2. Magnesium lamp. *A*, Quartz window sealed to glass envelope with black wax; *B*, glass envelope; *C*, tungsten anode; *D*, C70 glass-to-metal seal; *E*, sealed-off connexion to vacuum system; *F*, screw locking cathode in position; *G*, tungsten-rod support for cathode; *H*, hard-glass sleeve; *I*, hollow magnesium cathode.

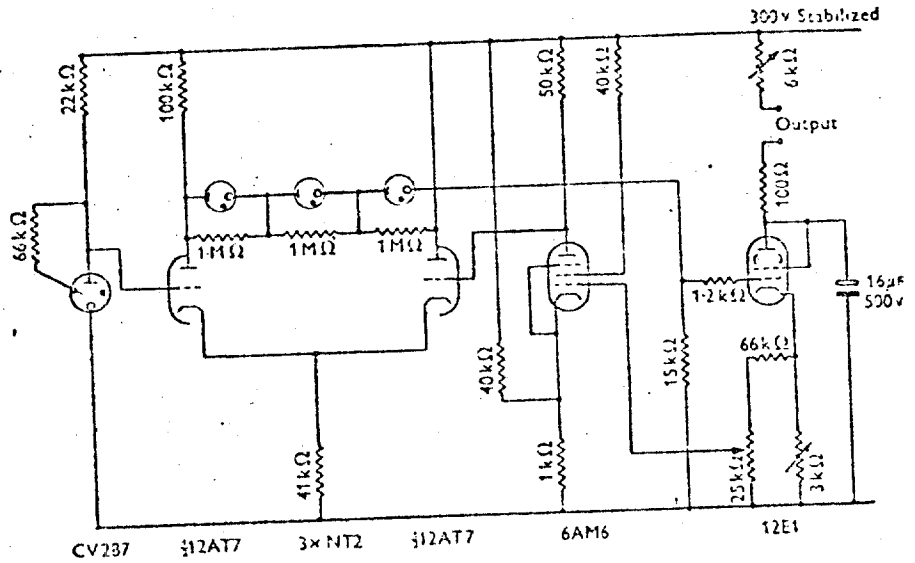

Fig. 3. Circuit diagram of current-regulated power supply for magnesium lamp.

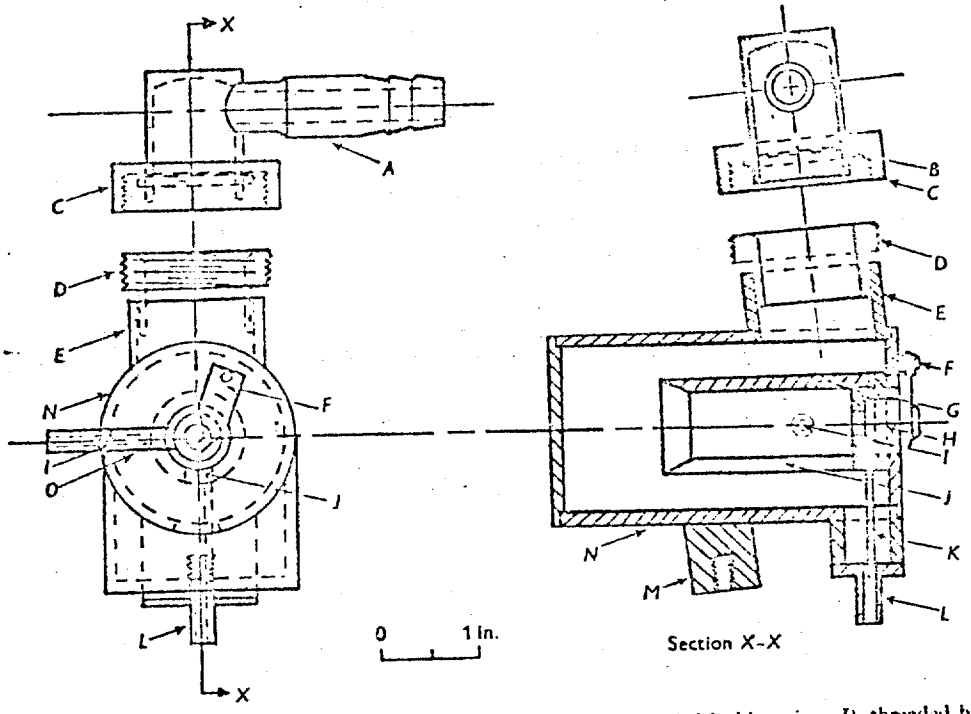

Fig. 4. Spray chamber. *A*, Connexion to burner; *B*, O-ring seal; *C*, knurled locking ring; *D*, threaded brass flange; *E*, Perspex tube at angle of 5° to main body; *F*, clip to secure EEL atomizer; *G*, inner Perspex cylinder; *H*, recess for atomizer; *I*, acetylene inlet; *J*, slit in inner cylinder for drainage; *K*, rod guiding drops to outlet; *L*, drain; *M*, block fastening chamber to bench; *N*, outer Perspex cylinder; *O*, groove to accommodate side arm of atomizer.

hollow magnesium-cathode discharge tube, filled with argon at a pressure of 1·2 mm. Hg (Fig. 2). Power is supplied to the lamp by a current stabilizer (Fig. 3), which is fed from a Solartron constant-voltage supply, type AS 517 (Solartron Electronic Group Ltd., Thames Ditton, Surrey).

Gas supply. Air and acetylene are supplied from cylinders fitted with two stage regulators (types BOR 12 and BAR 9 respectively, British Oxygen Gases Ltd., Wembley), fine control of the acetylene flow rate being provided by a needle valve situated on the instrument.

Atomizer. The atomizer is of the type used in the EEL flame photometer (Evans Electroselenium Ltd., Harlow, Essex).

Spray chamber. This is constructed of Perspex (Fig. 4), the drain running into a sealed bottle to help maintain a steady gas pressure in the chamber.

Burner. The burner, constructed of brass, is water-cooled to eliminate instability due to temperature variation (Fig. 5). A diaphragm is inserted in the light-path to restrict absorption measurements to the optimum part of the flame.

Monochromator and detection system. This consists of the monochromator and detection system of the CF4 spectrophotometer (Optica U.K. Ltd., Gateshead-on-Tyne), the lamp housing being replaced by a parallel-bar optical bench. On the bench are mounted the magnesium lamp, a condenser lens, the burner and a mirror which reflects the light into the entrance slit of the monochromator.

Preparation of the atomic absorption spectrophotometer

The positions of the lamp, lens and mirror are adjusted to pass maximum light into the monochromator (maximum transmission on the extinction scale). The wavelength control is set at 285 mμ and then finely adjusted to obtain maximum transmission at the magnesium resonance line 285·213 mμ. The slit width is set at 0·2 mm. The position of the burner is adjusted until the gas ports are aligned with the optical axis and 12 mm. below this axis. The instrument is checked to ensure that addition of sodium (17 mg./100 ml.) to a standard magnesium solution (0·05 mg./100 ml.) produces no change in extinction. If interference is observed, the height of the burner is adjusted relative to the optical axis until this effect disappears. The instrument is now correctly assembled, and further adjustment is necessary only after moving any part of the equipment.

The atomizer and gas ports in the burner should be cleaned after approximately 100 estimations, and the drain bottle from the spray chamber must be emptied periodically. No other routine maintenance is required.

Operation of the atomic absorption spectrophotometer

The magnesium lamp is allowed to stabilize for approximately 1 hr. before the instrument is used. Cooling water is then passed through the burner, the pressures of air and acetylene are adjusted to 13 and 5 lb./in.^{2} respectively, and, with the needle valve fully open, the gas is ignited. The acetylene flow is reduced by means of the needle valve until the white tips just disappear from the cones of unburnt gas in the flame. Water is aspirated into the flame and the sensitivity control is adjusted to give an extinction approaching zero. No attempt is made to adjust exactly to zero as it is impossible to stabilize completely the light output from the lamp.

Estimations may conveniently be carried out in groups containing up to eight unknown solutions. A standard solution containing 0·050 mg. of magnesium in 100 ml. of 0·1*N*-hydrochloric acid is measured immediately before and after each group of unknown solutions. The small effect of lamp instability is minimized by spraying water both before and after each magnesium-containing solution, the mean value of these two readings being subtracted from the extinction of the sample. The extinction of every solution is measured twice and, after correction for the distilled

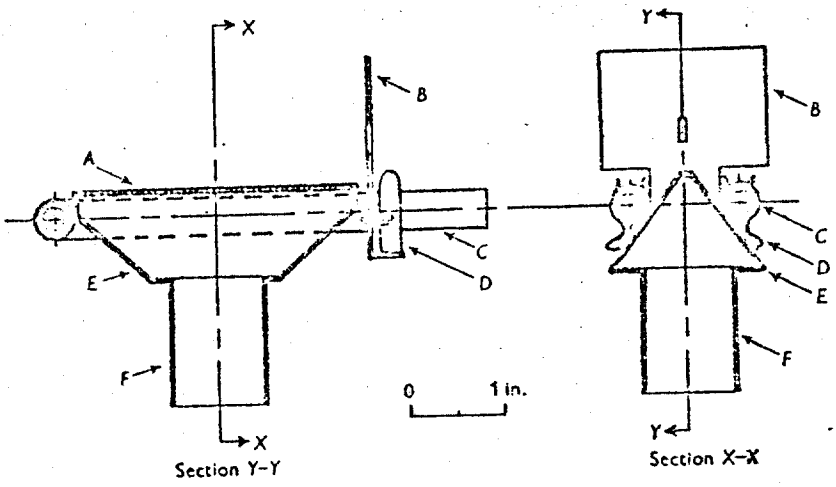

Fig. 5. Burner. *A*, Gas ports (30 × 1 mm. diameter); *B*, metal diaphragm; *C*, water-cooling pipes soldered to body of burner; *D*, Terry clips attaching diaphragm to cooling pipes; *E*, burner body fabricated from 16 s.w.g. sheet brass; *F*, connexion to spray chamber.

water blank, the magnesium content of each unknown is calculated from the ratio of the mean value for its extinction to the corresponding value for the standard solutions.

Preliminary treatment of samples

Specimens are diluted to produce a solution for measurement in the instrument which contains 0·02–0·05 mg. of magnesium/100 ml. Hydrochloric acid (2*N*) is added to each solution to produce a final concentration of 0·1*N*.

Plasma or serum. Direct, 50-fold dilution is satisfactory.

Urine. Dilution 100 times is usually satisfactory.

Blood cells. Packed cells are laked with a considerable excess of water before acidification; 100-fold dilution is satisfactory.

Food and faecal materials. Solid materials are homogenized with water and diluted to a known volume. Suitable portions are transferred to silica crucibles, dried on a sand bath and ashed overnight in a muffle furnace at 500°. The ash is dissolved in 2*N*-hydrochloric acid and diluted as described above.

Reagents

Chemicals. All materials used were of AnalaR quality with the exception of dipotassium hydrogen phosphate and ethylenediaminetetra-acetic acid (disodium salt), which were of laboratory-reagent grade. The standard calcium solution was prepared from dry calcium carbonate dissolved in the minimum quantity of hydrochloric acid. Glass-distilled water was used for the preparation and dilution of all solutions.

Standard magnesium solution. A stock solution of $MgSO_4,7H_2O$ containing 10 mg. of magnesium/100 ml. is employed. Working strength standards of 0·050 mg. of magnesium/100 ml. are prepared by dilution, and acidified with hydrochloric acid to 0·1*N* in the same manner as the solutions to be analysed. The working standards are stable for 1 week provided they are stored in flasks aged by equilibration with similar solutions.

Chemical estimation of magnesium

Magnesium was determined by the ammonium phosphate separation method of Simonsen, Westover & Wertman (1947) for serum magnesium, modified and extended to the analysis of other materials as described by Heaton (1960).

RESULTS AND DISCUSSION

Development of the method

Treatment of solutions. Magnesium sulphate and magnesium acetate, the two non-deliquescent magnesium salts readily available in a high state of purity, were examined for use as standards. A neutral aqueous solution of magnesium sulphate produced an absorption appreciably greater than that of an equimolar solution of magnesium acetate (Table 1). When these solutions were treated with acetic acid or sulphuric acid in quantities sufficient to equate their anionic composition, an increased extinction was observed in both cases, and the two solutions gave identical results. Similar agreement was obtained when solutions of both salts were acidified with an excess of hydrochloric acid.

Acidification with hydrochloric acid to a concentration of 0·1*N* was adopted as a routine procedure with both standard and unknown solutions. Under these conditions, a standard solution containing 0·050 mg. of magnesium/100 ml. produced an extinction of approximately 0·10, and the calibration curve was linear up to a magnesium concentration of 0·10 mg./100 ml.

Selection of correct region in the flame. The optimum part of the flame for use in atomic absorption measurements was immediately above the cones of unburnt gas. If the burner was too high relative to the optical axis, addition of other cations to a standard magnesium solution caused an apparent enhancement of the magnesium concentration (Table 2).

Evaluation of the proposed method

The evaluation of the atomic absorption method reported in this section was carried out with the height of the burner correctly adjusted, and all solutions were acidified with hydrochloric acid as described in the Experimental section.

Interference effects. (*a*) Inorganic ions. The major inorganic constituents of biological materials were tested for interference at the concentrations at which they normally occur, and at twice these levels. Solutions of suitable salts were added to solutions of known magnesium content, and the apparent magnesium concentration was deter-

Table 1. *Influence of anions on the absorption of standard magnesium solutions*

Solvent	Extinction for 0·050 mg. of Mg/100 ml.	
	Magnesium sulphate	Magnesium acetate
Distilled water	0·1103	0·1058
Acetic acid, 50 μ*N*	0·1149	—
Sulphuric acid, 50 μ*N*	—	0·1149
Hydrochloric acid, 0·1 *N*	0·1125	0·1121

Table 2. *Effect of burner height on interference produced by other cations*

The magnesium concentration was 0·050 mg./100 ml.

Distance of burner below optical axis (mm.)	Percentage interference		
	Sodium (17·0 mg./100 ml.)	Potassium (4·0 mg./100 ml.)	Calcium (0·50 mg./100 ml.)
4	+4·4	+3·7	+1·3
6	−0·2	+3·7	+0·7
8	+0·8	+2·2	+1·3
10	+1·2	+2·4	−0·4
12	0·0	+0·9	+0·1
14	+0·4	+0·8	+1·8

mined, making due allowance for the small blank readings of the added salts. The interference observed with different salts was random in nature (Table 3), varying from +1·0 to −1·6% of the magnesium concentration, and was equal to the experimental error of the measurement.

(b) Organic substances. The organic constituents of plasma, blood cells and urine were examined for interference by comparing the magnesium content, measured after simple dilution, with the values obtained after preliminary ashing of the same specimens. Since interference by inorganic ions is negligible the results obtained with ashed material were employed as a standard of reference. Organic material enhanced the apparent magnesium content of all types of specimen (Table 4). With urine and blood cells the enhancement of 0·4% was insignificant, but with plasma the effect was rather greater (2·3%).

For the determination of serum magnesium Willis (1960*b*) advocated dilution with solutions of either the disodium salt of ethylenediaminetetra-acetic acid or of strontium chloride to liberate the protein-bound magnesium, which normally constitutes approximately 35% of the total present in serum (Copeland & Sunderman, 1952; Prasad, Flink, Zinneman & McCollister, 1958). Acidification with hydrochloric acid, however, produced results similar to those obtained by these procedures (Table 5), and simple aqueous dilution yielded

Table 3. *Interference effects of inorganic ions*

The magnesium concentration was 0·0500 mg./100 ml.

Cation	Concn. (mg./100 ml.)	Anion	Concn. (mg./100 ml.)	Apparent Mg concn. (mg./100 ml.)	Percentage interference
Na^+	8·5	Cl^-	13·1	0·0498	−0·4
	17·0		26·2	0·0499	−0·2
K^+	1·96	PO_4^{3-}	2·30	0·0505	+1·0
	4·01		4·88	0·0502	+0·4
Ca^{2+}	0·25	Cl^-	0·44	0·0504	+0·8
	0·50		0·88	0·0499	−0·2
NH_4^+	0·76	SO_4^{2-}	2·02	0·0492	−1·6
	1·52		4·04	0·0493	−1·4
Fe^{2+}	1·0	SO_4^{2-}	1·71	0·0502	+0·4
	2·0		3·42	0·0498	−0·4
Fe^{3+}	1·0	SO_4^{2-}	1·71	0·0502	+0·4
	2·0		3·42	0·0502	+0·4

Table 4. *Comparison of magnesium analyses carried out by (a) direct dilution and (b) preliminary ashing of the same specimens*

Magnesium concn. (mg./100 ml.)					
Plasma		Urine		Blood cells	
(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
1·68	1·63	3·11	3·18	4·81	4·95
1·80	1·73	2·79	2·86	6·46	6·27
1·71	1·71	3·40	3·49	4·48	4·35
1·97	1·94	9·85	9·70	5·67	5·78
1·68	1·61	7·47	7·47	6·07	6·12
2·00	2·01	5·51	5·41	5·52	5·43
Mean 1·81	1·77	5·36	5·34	5·50	5·48

Table 5. *Effect of various diluents on analysis of plasma*

Plasma diluent	Extinction: Mg standard (0·050 mg./100 ml.)	Extinction: Plasma (diluted 50 times)	Plasma Mg concn. (mg./100 ml.)
Distilled water	0·1161	0·0920	1·98
Hydrochloric acid, 0·1 N	0·1185	0·0910	1·92
Ethylenediaminetetra-acetic acid, disodium salt, 10·0 g./l.	0·1156	0·0900	1·94
Strontium chloride, 7·6 g./l.	0·1127	0·0872	1·93

results 2·5% higher. It appears therefore that displacement of the protein-bound magnesium either occurs during aqueous dilution or is not necessary for the atomic absorption method, and the substances used by Willis, in relatively large concentrations, may be acting as spectroscopic buffers in a manner similar to hydrochloric acid.

Reproducibility and speed. The average difference between duplicate determinations of the extinction on 40 different solutions was 1·0%. Duplicate estimations of the magnesium content of 20 different specimens varied by an average of 1·5%. This larger error includes both the instrumental error and the volumetric errors involved in the preparation of solutions for assay. After preparation of the solution a single extinction can be measured in approximately 1 min., whereas duplicate measurements of extinctions on a group of ten solutions require 20–30 min.

Table 6. *Comparison of the atomic absorption and magnesium ammonium phosphate methods*

Plasma Mg (mg./100 ml.)		Urine Mg (mg./100 ml.)	
Atomic absorption	Phosphate separation	Atomic absorption	Phosphate separation
1·87	1·78	5·44	5·33
1·92	1·93	3·11	3·07
1·81	1·75	5·73	5·53
1·64	1·68	3·39	3·27
1·74	1·71	2·79	2·83
1·61	1·68	5·32	5·14
1·80	1·81	3·09	2·99
1·68	1·69	10·30	10·80
1·83	1·80	7·44	7·29
1·66	1·59	1·86	1·69
2·08	2·00	6·05	5·77
1·97	2·02	5·11	5·18
2·24	2·09	2·67	2·40
1·96	1·95	5·40	5·58
1·71	1·67	2·67	2·65
1·80	1·73	4·86	4·88
1·68	1·63	3·40	3·40
1·96	2·00	9·56	9·66
Mean 1·84	1·81	4·91	4·86
Mean difference 0·03 (S.E. 0·01)		Mean difference 0·05 (S.E. 0·04)	

Food or faecal ash Mg (mg./100 ml.)		Blood cell Mg (mg./100 ml.)	
Atomic absorption	Phosphate separation	Atomic absorption	Phosphate separation
6·90	7·10	5·52	5·28
4·81	4·80	5·67	5·77
5·99	6·19	6·46	6·15
4·54	4·29	4·48	4·37
5·41	5·64	4·62	4·82
4·67	4·64	5·39	5·53
Mean 5·30	5·44	5·36	5·32
Mean difference 0·05 (S.E. 0·07)		Mean difference 0·04 (S.E. 0·08)	

Comparison of the atomic absorption and magnesium ammonium phosphate methods. A micro-modification of the classical ammonium phosphate method has been used as a routine in this laboratory for the determination of magnesium. The principle of this method is the precipitation of magnesium as the ammonium phosphate salt, the magnesium present in the precipitate being calculated from the phosphate content as determined colorimetrically. Calcium is previously removed by precipitation with ammonium oxalate.

Simultaneous analyses were made on 120 different specimens by the proposed method and the ammonium phosphate separation procedure. Typical results are shown in Table 6. The atomic absorption method produced slightly higher results than the chemical method except with inorganic solutions of food and faecal ash, where the average value for the ammonium phosphate method exceeded that for the atomic absorption procedure by 0·9%. With blood cells, urine and plasma the mean values obtained by the proposed method were respectively 0·8, 1·0 and 1·7% higher than the corresponding results from the chemical method.

Accuracy of the magnesium ammonium phosphate method

The systematic errors inherent in this method can be divided into two groups: those which decrease the magnesium content of the final precipitate, and compensatory errors leading to the presence of an enhanced amount of phosphate in the precipitate. The greater sensitivity of the atomic absorption method was used to determine the magnitude of these errors. The magnesium loss was ascertained by determining the total magnesium present in the calcium oxalate precipitate, the supernatant fluid from the magnesium ammonium phosphate precipitate, and the liquid used to wash this precipitate free from excess of phosphate. The magnesium equivalent of the phosphate gain was determined by comparing the magnesium content of the magnesium ammonium phosphate precipitate, as measured directly by atomic absorption spectrophotometry, with the corresponding value obtained by calculation from the phosphate content of this precipitate measured colorimetrically. Suitable blank solutions were measured, and allowance was made for trace contamination introduced by the reagents employed in the chemical procedure. Most of the measurements were carried out on the analyses of serum and urine reported in Table 6.

A total magnesium loss of 2·2–2·5% was observed with both types of material, and this was usually balanced by the phosphate gain, giving results within ± 1% of the true value (Table 7). As

Table 7. *Systematic errors in the magnesium ammonium phosphate method*

Each value is the mean of 20 estimations.

Material	Magnesium loss (%)	Phosphate gain (%)	Net error (%)
Serum or plasma	2·5 (S.E. 0·5)	1·7 (S.E. 0·4)	−0·8
Urine	2·2 (S.E. 0·4)	2·3 (S.E. 0·6)	+0·1

would be expected, however, the phosphate gain was proportional to the magnesium concentration, which is normally greater in urine than in serum. For serum this gain was slightly less than the magnesium loss and so caused underestimation of the magnesium content by 0·8%, but with urine the average phosphate gain exceeded the magnesium loss very slightly, producing results 0·1% high. As the procedures for the analysis of blood cells and solutions of ashed material are identical with the methods for serum and urine respectively, the errors inherent in the latter analyses may be considered typical of those occurring during analysis of the former materials.

Occasionally larger and non-reproducible errors were encountered, owing to disturbance of the magnesium ammonium phosphate precipitate during manipulation. Estimations where such errors occurred were excluded in the compilation of Tables 6 and 7.

Absolute accuracy of the atomic absorption method

Direct comparison with the ammonium phosphate procedure showed that the atomic absorption method produces results 0·8, 1·0 and 1·7% higher than the chemical method with blood cells, urine and plasma respectively, the mean value for ashed material being 0·9% below that given by the chemical procedure. If allowance is made for the errors in the chemical method, the results obtained by atomic absorption spectrophotometry for plasma, urine and food or faecal ashes differ respectively by +0·9, +1·1 and −0·8% from the correct values. These results are not in exact agreement with the probable errors indicated by interference studies, the discrepancy varying from 0·4% with blood cells to 1·4% with plasma. No attempt was made to resolve these differences by studying larger series of analyses, as they are sufficiently small to be without practical significance, and do not affect the conclusion that the atomic absorption method is accurate with all types of material to within 2% of the absolute value.

SUMMARY

1. An instrument suitable for the determination of magnesium by atomic absorption spectrophotometry is described.

2. Analyses may be carried out on serum, urine, blood cells and solutions of the ash from food or faecal materials after simple dilution and acidification with hydrochloric acid.

3. No significant effect is produced by other inorganic ions. The organic constituents of samples cause slight overestimation of the magnesium concentration.

4. The magnesium ammonium phosphate method is examined and its accuracy determined.

5. Comparison between analyses carried out by the ammonium phosphate method and the atomic absorption procedure shows the latter to be accurate to within 2% of the absolute value.

We wish to thank Mr D. J. Ellis, Mr S. Taylor and Mr A. W. Chivers for their invaluable help with the construction and evaluation of the instrument.

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Notes

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

AN UNUSUAL ADULTERANT FOR PEPPER

FROM one of our Authorities we recently received four sealed samples of pepper which we will designate A, B, C and D; the last was described as "Pepper" and the others as "White Pepper." Microscopically, they appeared to be perfectly genuine; D contained more husk than the others.

The ash of these samples we found to be 4·29, 1·02, 0·98, and 4·70 per cent., respectively, and when re-carbonated they were 8·28, 1·24, 1·22, and 8·78 per cent.

We examined the ash of samples A and D, in both of which we found notable amounts of magnesia, which we determined quantitatively, and found in A 7 per cent. and in D 8 per cent. (calculated as $MgCO_3$).

The two samples were further examined and found to contain magnesium carbonate. This adulterant, in these amounts, is difficult of detection, as it is likely to be missed in the microscopical examination, and, unless the ash is re-carbonated, its amount might not arouse suspicion, particularly if the sample be described as "pepper."

The appearance of these two samples was excellent—that of white peppers in fact, but on removing the magnesium carbonate by dilute acids, the original pepper in D was seen to be distinctly inferior to that in A, as containing considerably more husk.

Not only does the addition of magnesium carbonate improve the appearance of the pepper, but its employment presents some temptation, as its cost is about one-third that of pepper.

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THE PHOSPHATASE TEST

WE have been carrying out the phosphatase test upon samples of pasteurised milk for two local authorities in London for the last six months.

So far as our results indicate, we have found the test extremely useful, not only in detecting gross errors in pasteurisation, but also in revealing small errors in technique—such as insufficient period of heating (less than half-an-hour) or heating slightly below 145° F.. Recently we have had an interesting border-line case which clearly indicates the utility of the test in this direction. A particular sample gave a blue value of 3·3 Lovibond units and we reported that the results indicated a slight error in technique. The sanitary inspector, therefore, checked the temperature records of the particular dairy in question, and he was able to ascertain that on that particular day the temperature of pasteurisation was near 140° F. for a considerable time.

Regarding the method of carrying out the test, we have also noticed a number of points emphasised in the paper by Anderson, Herschdörfer and Neave (see p. 86). There is one point, however, about the "buffer substrate" which is worth noting. We have used, throughout, tablets supplied by the British Drug Houses, and have found them quite satisfactory. These tablets are particularly useful when one has to make a few tests intermittently, and not regularly from day to day, because a

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SIMPLIFIED METHODS FOR DETERMINATION OF CALCIUM AND MAGNESIUM IN THE SALIVA

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THIS article describes the application to saliva of two analytic methods: the titration of calcium and magnesium with disodium ethylene diamine tetraacetic acid, and the colorimetric determination of magnesium by the titan yellow procedure. Observations made during the work which seem to be of more general interest are also reported.

I. Calcium and Magnesium Determination by Complexone Titration

Calcium, magnesium, and other metals are firmly bound in un-ionized complexes by certain organic compounds which have been called complexones. Certain dyes have a similar, although weaker, metal-binding capacity. If the color of such a dye is distinctly different from that of its complex with a metal, it can be used as an indicator for titration of the metal ion in question with a complexone.

Techniques based upon this principle have been rapidly developed during the last few years. For determination of the alkaline earth metals, disodium ethylene diamine tetraacetate (EDTA) has been applied most often; a basic paper on this subject is that of Biedermann and Schwarzenbach.[2]

In the biologic field, methods have been evolved to determine calcium in plasma and in serum, urine, and spinal fluid[15] by titration with EDTA, using murexide (ammonium purpurate) as indicator. Another technique uses Eriochrome Black T as indicator for Mg ions. This dye shifts from red to blue when all Mg ions have been bound by the EDTA titration solution. It can hardly be used for titrations of Ca alone, because its color shift is then too gradual and indistinct. If Ca and Mg are present simultaneously, however, Ca is bound first by the EDTA, and a sharp end point is obtained in the Mg range. The total amount of Ca and Mg can thus be determined; if Mg is determined in a separate sample after the Ca has been precipitated as the oxalate, Ca is obtained as the difference. Such methods have been applied mainly in water analysis,[5, 13] but also for determinations in biologic fluids.[9]

A third method, used for ultra-micro estimations on serum,[11] involves the same determination of Ca and Mg, followed by the separate determination of Mg by a special colorimetric method[27]; Ca is obtained as the difference.

ORIGINAL INVESTIGATION

Various modifications of the methods outlined previously were tested with reference to suitability of calcium and magnesium analysis in the saliva. The

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technique finally adopted was found to be about as accurate as, and much more convenient than, the method[20] previously used by the present author, the principles of which were: precipitation of Ca as the oxalate, conversion into the carbonate by ignition, and titration of the carbonate with hydrochloric acid. Lazarev[23] described this procedure as the most accurate of established techniques for calcium analysis.

The use of murexide as an indicator for direct calcium titration was found impracticable, because the color shift was much too weak and indistinct to the investigator's eye; nor were efforts with spectrophotometric reading of the color shift successful. The work was therefore concentrated on the titration of Ca and Mg with Eriochrome Black T as indicator, and the separate determination and deduction of Mg.

The EDTA titration was thoroughly tested with known salt solutions before being applied to the saliva. The general procedure is described in the following:

The EDTA titration must be performed in an alkaline buffer of pH about 8 to 10, since the indicator Eriochrome Black T is also a pH indicator, having a wine red color below pH 6.3 and an orange color above pH 11.5. The indicator is sensitive not only to Ca and Mg ions but also to a great number of other cations.[2, 3] None of these is, however, present in the saliva except as traces, e.g., of Cu and Fe.

An important observation was made when different concentrations of Mg standard in the range 0.1 to 2.0 mM per liter were titrated: the EDTA consumption is not proportional to the Mg concentration, being comparatively higher at lower concentrations. The reason for this phenomenon seems to be indicated by the fact that a higher indicator concentration gave a greater deviation from proportionality. The indicator Eriochrome Black T probably binds an appreciable fraction of a low Mg concentration.[2] Since the deviation was found to be linear, at least up to 2 mM Mg per liter, there is no difficulty in preparing a calibration graph for a given buffer-indicator mixture. Fig. 1 shows a typical graph of this type for one such mixture.

The determination of Mg alone by this method required quantitative removal of Ca. The classical method of precipitation as the oxalate was tested with known standard solutions of concentrations approximating those found in the saliva. A serious complication was the frequently incomplete precipitation of Ca as the oxalate. Recent investigators have encountered this difficulty.[11, 23, 24] With different techniques for precipitation of small concentrations of Ca these authors found 0.015-0.3 mM Ca per liter unprecipitated.

In view of the importance of the oxalate precipitation for the determination of both Ca and Mg in saliva and other biologic fluids, a fairly detailed study was undertaken with salt solution concentrations approximating those of the saliva, and using such small volumes as can easily be obtained, in the case of saliva, without stimulation. Mg, or Mg and unprecipitated Ca, was determined by EDTA titration using the technique described in detail at the end of this section, in some of the experiments with slight modifications. The standard solutions used were mixtures of $CaCl_2$ and $MgCl_2$, of fixed concentrations be-

tween 1 and 2 mM Ca and 0.1-0.5 mM Mg per liter. In some tests phosphate was added to the concentration of 5 mM per liter, and HCl to a reaction that prevented precipitation and gave a pH of 4.3 on the subsequent addition of 0.2 volume of saturated ammonium oxalate.

No difference could be detected between precipitations at different pH values between 4 and 7, and no coprecipitation of Mg was ever found. This was in agreement with Holth's observation that magnesium oxalate was never precipitated from solutions of concentrations below 3 mM Mg per liter.[19]

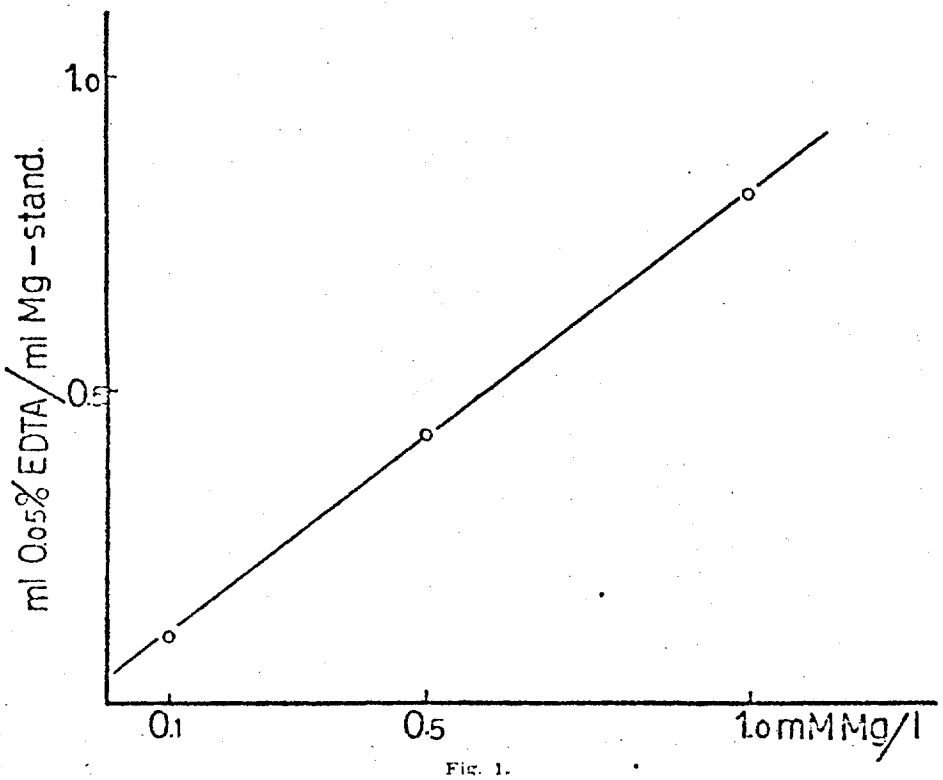

Fig. 1.

The concentrations of unprecipitated Ca were generally of the order, 0.01 to 0.02 mM per liter. No differences were obtained with varying precipitation times exceeding one-half hour. This is apparent from Table I, which shows the results of two typical series of tests in which the titrations were performed by the technique given later. In one of these series, as in some other tests, precipitation was also carried out at 80° C. overnight, followed by several hours' refrigeration; no more Ca was removed. As Table I shows, the different centrifugation periods tested gave no consistently different results; neither did decantation, compared with a suction technique that prevented swirling, or removal of any surface film. In a number of series, these separation techniques were found more reliable than filtration through even the best paper filters or the finest glass filters.

TABLE I

TESTS ON CALCIUM OXALATE PRECIPITATION

PRECIPITATION CONDITIONS	SER. A				SER. 2	
	CENTRIFUGATION 5 MIN.		CENTRIFUGATION 15 MIN.		CENTRIFUGATION 5 MIN.	CENTRIFUGATION 15 MIN.
	DECANT.	SUCTION	DECANT.	SUCTION	DECANT.	DECANT.
30 min., room temp.	0.145	0.156	0.156	0.168	0.162	0.144
3 hours, room temp.	0.160	0.165	0.168	0.165	0.150	0.162
Overnight, room temp.	0.168	0.156	0.156	0.162	0.168	0.168
Overnight, 80° C., + refrig. 4 hours	----	----	----	----	0.168	0.168
	Med. = 0.160				*Med.* = 0.161	

Two milliliters salt solution (1.5 mM Ca, 0.15 mM Mg, 5 mM total phosphate per liter) + 0.4 ml. saturated $(NH_4)_2C_2O_4$. All centrifugations at about 2,000 r.p.m. Duplicate titrations with 0.05% EDTA solution.

Since the concentration of unprecipitated Ca occasionally reached the order of 0.1 mM per liter, this method could not be regarded as satisfactory, especially for determination of the low Mg concentrations generally occurring in the saliva, 0.15 to 0.25 mM per liter. As a result of this, efforts were made to determine the salivary Mg by the colorimetric titan yellow method.

In the *application of the EDTA titration to saliva*, it seemed essential to ascertain that no disturbances were introduced by its organic constituents or by the Ca complex formation probably occurring in this fluid.[5,6] Comparative titrations were, therefore, made with saliva both directly and after ashing, the ash being dissolved in 0.1 N HCl to the original volume. It was found that ashing gave consistently higher titers for Ca and Mg, the differences being from 3 to 15 per cent. In addition, spurious end points were frequent in the direct saliva titration. The blue end-point color changed back to violet after some minutes, indicating the liberation of new Ca or Mg ions. Since this might be supposed to take place either from organic or inorganic complexes or from suspended solid particles, it seemed logical to expect an effect from direct acidulation similar to that obtained by ashing with subsequent dissolution of the salt in acid.

Acidulation to the saliva's isoelectric range of pH 2.5 to 3.0 can be effected by adding 0.3 volume 0.1 N HCl. It was found, however, that titration after this treatment gave values between those obtained by the direct titration and ashing procedures. On the other hand, if the common precipitation with trichloroacetic acid was performed by adding 0.3 volume of 10 per cent acid, the recovery was the same as, or somewhat higher than, with ashing. The same results were obtained by precipitation with 0.3 volume normal HCl, which gave about the same pH level (slightly above 1) as trichloroacetic acid. These data are given in Table II.

Control experiments with the addition of trichloroacetic acid in the same proportion to a known salt solution resembling saliva gave no change of the titer. Since, moreover, the agreement between trichloroacetic acid and normal hydrochloric acid treatment was good, these techniques were accepted as giving the most accurate results. No definite explanation can be offered for the lower values found after ashing, but it is known that magnesium ammonium phosphate

in particular is gradually transformed into the pyrophosphate on ignition. In solution the pyrophosphate is changed back to the orthophosphate rather slowly, and it might thus reduce the titer by its strong binding action on Ca and Mg.

Recommended Technique for Determination of Ca and Mg in Saliva.

Solutions (all of reagent grade chemicals in distilled water).—

Ammonia Buffer, pH about 10: 1 vol. M NH_4Cl + 7 vol. M NH_4OH.

Indicator solution: 25 mg. Eriochrome Black T (Eastman Kodak No. P6361) dissolved in 50 ml. ethanol. Stored in refrigerator.

Buffer-indicator mixture: 1 ml. of indicator solution added to each 15 ml. of ammonia buffer. A sufficient volume for titration of the whole analytic series and the necessary Mg standards is made up within a few hours of the titration, because the color is unstable over long periods.

TABLE II

RESULTS OF EDTA TITRATIONS OF SALIVA*

EXP. NO.	ASHING (1)	CCl_3COOH (2)	N HCl (3)	DIFFERENCES (2-1)	(3-1)	(3-2)
1	1.19	1.19		0.00		
2	1.35	1.35		0.00		
3	1.10	1.12		+0.02		
4	0.92	0.82		−0.10		
5	1.24	1.24		0.00		
6	1.34	1.39		+0.05		
7	1.20	1.26		+0.06		
8	0.92	0.99		+0.07		
9	1.18	1.27	1.27	+0.09	+0.09	0.00
10	1.26	1.32	1.32	+0.06	+0.06	0.00
11	1.11	1.18	1.14	+0.07	+0.03	−0.04
12	1.18	1.24	1.20	+0.06	+0.02	−0.04
13	1.15	1.25	1.24	+0.10	+0.09	−0.01
14	1.14	1.22	1.21	+0.08	+0.10	+0.02
15	1.26	1.58	1.33	−0.12	+0.07	−0.05
16	1.22	1.32	1.32	−0.10	+0.10	0.00
17	1.26	1.32	1.32	−0.06	+0.06	0.00
18	1.31	1.25	1.28	−0.06	−0.03	+0.03
Mean differences		No. 1-8		+0.013		
		No. 9-18		+0.068	+0.059	−0.009
		No. 1-18		+0.043		

*After (1) ashing at 650° to 700° C.; ash dissolved in original volume of 0.1 N HCl, (2) protein precipitation with 0.3 vol. 10 per cent CCl_3COOH, and (3) protein precipitation with 0.3 vol. normal HCl. All concentrations are in millimols per liter.

EDTA solution, 0.05 per cent: 250 mg. disodium ethylene diamine tetra-acetate dihydrate dissolved in 500 ml. water and stored in a Pyrex bottle. Stable for months.

Standard solutions of $MgCl_2$: The titer of an approximately 100 mM $MgCl_2$ solution is determined exactly, e.g., by titration with $AgNO_3$ using dichlorofluorescein as indicator.[22] Strengths of 0.5, 1.0, 1.5, and 2.0 mM per liter are prepared from this stock solution. A drop of toluene is used as preservative.

Procedure.—Mix 1 ml. saliva and 0.3 ml. normal HCl (or 10 per cent trichloroacetic acid) in a centrifuge tube. After one-half hour, centrifuge and decant. Pipette 0.5 ml. volumes in duplo into 12-ml. round-bottomed Pyrex tubes. To each tube add 8 ml. buffer-indicator mixture. Pipette into a similar tube 1.5 ml. distilled water and 8 ml. buffer-indicator mixture. Titrate the

saliva samples with the EDTA solution to a color matching that of the blank tube. Use a burette with immersing capillary point and graduated in 0.01-0.001 ml.; provide a good light source behind the tubes; introduce air bubbling to mix the contents of the titration tube. Ascertain the end-point reading by subsequent overtitration by 0.005 ml.; this will give a more distinctly blue color than the blue of the blank tube. Prepare a linear calibration graph by titrating one standard on each side of the concentration of the saliva samples, using the same buffer-indicator mixture. Read the concentrations of the saliva samples from this graph. Reading times 1.3 = salivary Ca and Mg.

A precision of 1.5 per cent has been obtained with a number of duplicate analyses by this technique.

II. Magnesium Determination by the Titan Yellow Method

If Mg is precipitated as the hydroxide in strongly alkaline solutions of certain dyes—titan yellow, thiazol yellow, brilliant yellow, Clayton yellow—the light yellow color shifts to red. These dyes apparently act as adsorption indicators for $Mg(OH)_2$. Sedimentation of the precipitate can be prevented and the color thus stabilized by the addition of protective colloids, such as starch, gum ghatti, agar, or polyvinyl alcohols.

Early workers who did not use protective colloids had great difficulty with the color instability due to sedimentation.[12, 21] Even with colloids difficulties arose from interfering metals (especially Ca, Al, Fe, and Mn), and phosphate ions. Several authors have tried to neutralize the interference by using compensation solutions containing these ions in high concentrations.[6, 22, 23, 24] A high concentration of polyvinyl alcohol was also used in order to prevent the precipitation which seemed to be the mechanism of these interferences.[24]

In the body fluids interference might be expected primarily from Ca and (PO_4) ions. Previous workers have made conflicting statements on this point, due possibly to their different techniques.[10, 17, 18, 27]

ORIGINAL INVESTIGATION

The titan yellow preparation used in all tests and experiments leading to the technique finally adopted was Eastman Kodak No. P4454. Differences have been reported between the products of various manufacturers.[26] Three polyvinyl alcohols were used: Du Pont's Elvanol 72-51, 51-05, and 70-05. The last grade was preferred since on standing it gave quite a clear solution with no sedimentation.

Solutions of polyvinyl alcohol, titan yellow, and sodium hydroxide were added, in this order, to the samples or standards which had been pretreated as will be described. Different proportions and concentrations were tested, departing chiefly from 3 of the works quoted previously.[10, 17, 27] In conformity with previous reports,[10, 18] it was found that the color development and the agreement with Beer's law were closely dependent on the titan yellow concentration. The variations in color development between different analysis series were found to necessitate the processing of at least 3 known standards along with each series.

Protein precipitation, with 1 volume 10 per cent trichloroacetic acid to 3 volumes of saliva, gave the well-known opalescent centrifugate. On the final addition of the sodium hydroxide solution, the opalescence disappeared. At the same time, however, an increasingly flocculent precipitate appeared which gave rise to rapidly increasing transmittance readings. This was evidently a calcium phosphate precipitate; analysis series with Mg standards in the presence of Ca and inorganic phosphate at about saliva concentrations gave the same precipitates, while these Ca or phosphate concentrations separately had no influence. It seems appropriate, therefore, to judge the influence of these ions on the titan yellow method from their combined effect and not from tests with each ion separately, as has been done in some previous works.

If the calcium of the saliva samples was removed as the oxalate, no precipitation occurred on the addition of sodium hydroxide, and stable transmittance readings were obtained. When the oxalate precipitate was filtered off, it was noticed that most of the proteins were removed at the same time without special measures for their precipitation; only a very slight opalescence remained sometimes, and this disappeared on the final addition of sodium hydroxide. Oxalate precipitation and filtration was, therefore, chosen as pretreatment of saliva for this analysis.

RECOMMENDED TECHNIQUE

Solutions.—

Ammonium oxalate, saturated solution: Add 6 Gm. of reagent grade $(NH_4)_2C_2O_4 \cdot H_2O$ to each 100 ml. of distilled water, heat to near boiling, and allow to cool.

Polyvinyl alcohol, 0.1 per cent: Dissolve 1 Gm. Du Pont Elvanol, grade 70-05, in 1 L. distilled water while heating on water bath and stirring vigorously. Preserve with some drops of toluene.

Titan yellow, 0.01 per cent: Dissolve 50 mg. titan yellow in 500 ml. distilled water. Store in dark bottle in refrigerator.

Sodium hydroxide, 15 per cent: Dissolve 45 Gm. reagent grade NaOH in 300 ml. distilled water.

Procedure.—To 1 ml. saliva, add 0.5 ml. oxalate solution. Mix. Filter after one-half hour. Pipette in order into a Coleman tube, 19 mm. diameter: 1 ml. filtrate, 1 ml. distilled water, 1 ml. polyvinyl alcohol solution, 1 ml. titan yellow solution, and 1 ml. NaOH solution. Mix by shaking between the additions; the water and reagents may be conveniently and accurately added by syringe pipettes.[21] Include blank, 0.1, 0.2, and 0.3 mM Mg standards in each analysis series. Read in spectrophotometer at 520 mμ the wave length of maximum absorption.

Results Obtained With Recommended Technique.—Ten consecutive determinations on a known salt solution containing 0.15 mM Mg per liter, and calcium and phosphate of about salivary concentration gave the mean value 0.163 mM with the standard deviation 0.007 mM. This result, 0.013 mM above the theoretical value, is representative of a greater number of tests carried out with different known solutions. A similar series with saliva gave the values: mean 0.14 mM, standard deviation 0.01 mM.

Ten tests with 0.15 mM Mg. per liter added to saliva gave the mean recovery 0.147 mM, standard deviation 0.021 mM.

No comparison was made with earlier methods for the determination of salivary Mg after precipitation as $MgNH_4PO_4$. It has never been established that such precipitates have the theoretical composition. The limitations of these methods have been clearly recognized by 2 investigators.[3]

Twenty-five analyses on individual samples of stimulated saliva revealed Mg contents varying between 0.13 and 0.23 mM per liter, with the mean value 0.18 mM per liter. Previous authors, using precipitation of $MgNH_4PO_4$, have all given higher average values, for several tested groups about 100 per cent higher, and greater variations.[4, 5, 21] Twenty-nine analyses of resting salivas gave the mean concentration 0.228 mM per liter, standard deviation 0.055 mM per liter.

COMMENT

The incomplete calcium oxalate precipitation at concentrations of the order found in saliva may be regarded as established by the papers quoted and the findings presented in this paper. The complexone titration is also limited by this difficulty as far as the separate determination of Mg is concerned.

The titan yellow method cannot be regarded as ideal but seems to be better than earlier methods. Of these, precipitation as $Mg\,NH_4PO_4$ evidently requires a much more elaborate technique for reliability than has generally been used.[4, 22] Although Ca determination by the methods presented here requires 2 different procedures, it is still more convenient than the methods based on analysis of an oxalate precipitate. For clinical estimations on saliva, e.g., in cases of excessive caries or the formation of calculus or salivary duct stones, a single titration of Ca and Mg and deduction of the average Mg content seems quite satisfactory, since the salivary Mg shows rather small variations. For a modified Fosdick test,[8a] the direct complexone titration of salivary Ca and Mg should be almost ideal.

SUMMARY

Two analytic methods for small saliva samples are presented:

1. Determination of the sum of calcium and magnesium by titration with ethylene diamine tetraacetic acid (EDTA), using Eriochrome Black T as indicator.

2. Determination of magnesium by the colorimetric titan yellow method. The analyses revealed lower values for salivary magnesium than have been previously reported.

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Diss. Abstr. Int. Sect. B. Sci. Eng., 30(2):696-697, 1969

amount of residue. It was concluded that: the toxicity of Rabon is fairly low for hens, Rabon did not inhibit egg production when fed to hens in quantities less than 100 mg/kg, it has high affinity for fatty tissues and amounts of 25 mg/kg did leave residues in fat, amounts up to 200 mg/kg did not leave any residues in liver, leg and breast muscles, Rabon was metabolized or eliminated from the body tissues within a period of seven days and eggs laid after that did not contain Rabon residues.

1. Rabon is 2-Chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate, a product of Shell Chemical Company.

M $3.00; X $4.20. 79 pages

FOOD TECHNOLOGY

OLFACTORY ADAPTATION AND DIRECT SCALING OF ODOR INTENSITY

(Order No. 69-9947)

William Stanley Cain, Ph.D.
Brown University, 1968

In the first part of the investigation a group of 15 subjects used magnitude estimation to scale the subjective intensity of five different odorants (acetone, geraniol, n-propanol, n-butanol and n-pentanol). Concentration was controlled by means of an air-dilution olfactometer and the subjects judged seven concentrations of each odorant. In accordance with predictions there was virtually perfect rank order correlation between the exponents of the psychophysical power functions and the water solubilities of the five odorants. These results need to be extended to other odorants and possibly offer a means of further specifying the critical physical parameters controlling discrimination of odor intensity.

The second part of the investigation concerned the scaling of odor intensity under conditions of self-adaptation. N-propanol and n-pentanol were chosen for study. A group of 19 subjects scaled each of these odorants after being exposed to an adapting stimulus which varied in intensity and duration from session to session. The psychophysical functions increased in steepness under adaptation conditions and, as expected, increases in the intensity of the adapting stimuli led to increases in the steepness of the functions. Of more importance was the finding that the psychophysical functions changed very little as the subjects exposure to any fixed adapting concentration was increased from three to as many as fifteen breaths. These results suggest that the decrease in suprathreshold intensity which occurs with increasing duration of exposure to an adapting stimulus has been overestimated in the past. This overestimation has undoubtedly been due to a response bias inherent in the time-course method previously used to study the problem.

The second experiment also provided evidence that the subjective intensity of an adapting stimulus determines the extent of self-adaptation irrespective of the odorant involved. Thus, subjectively matched concentrations of propanol and pentanol were found to have equal self-adapting effectiveness. The evidence for this effect was overwhelming but could only be seen when differences between the original non-adaptation psychophysical functions of propanol and pentanol were taken into account.

The last part of the investigation explored cross-adaptation between propanol and pentanol. Using the same basic procedure as in the second experiment it was found that pentanol has a strong cross-adapting effect on propanol, almost as strong as the adapting effect of propanol on itself. However, propanol had only a small cross-adapting effect on pentanol. Thus, subjectively matched concentrations of the two odorants were found to have quite unequal cross-adapting effectiveness. The results strongly illustrate the asymmetric effects of cross-adaptation within pairs of odorants, an asymmetry which must be considered in any attempt to use cross-adaptation as a basis for classification of odor quality.

M $3.00; X $7.40. 160 pages

STABILITY OF CHLOROPHYLL AND DERIVATIVES IN PROCESSED GREEN VEGETABLES

(Order No. 69-12,820) X $11.25

Daniel Leonard Fleischman, Ph.D.
University of Massachusetts, 1969

Adviser: Dr. F. J. Francis

To prevent the degradation of green chlorophyll to brown pheophytin during thermal processing and storage of puréed green vegetables, an attempt was made to produce a large amount of the postulated more stable green compound, chlorophyllide.

In order to increase the amount of chlorophyll to chlorophyllide conversion in spinach, a foreign source of the natural esterase, chlorophyllase, was added to the spinach purée mixture, in the form of beet greens. The beet greens, however, did not aid in increasing the amount of chlorophyllide produced.

When sodium phosphate was added as a buffering agent, and the final mixture adjusted to pH 8.0, an increase in the production of chlorophyllide was noted over samples which were buffered at different pH levels, or were not buffered. Magnesium carbonate, in conjunction with the sodium phosphate buffer, increased the chlorophyll to chlorophyllide conversion rate. At a 1.0% sodium phosphate buffer level, an almost equal amount of chlorophyll to chlorophyllide conversion was noted as at a higher buffer concentration, and was greater than at lower buffer levels.

Certain surface-active agents, when added to spinach purée, exerted a protective effect on the chlorophyll within the spinach. The agents prevented chlorophyll degradation at temperatures up to 212°F. However, the protective effect of these agents was not evident at thermal processing temperatures of 240°F.

Over a 12 week storage period at 100°F (37.8°C.) in the dark, the color of spinach which had been processed at 240°F. and buffering with sodium phosphate did not show much improvement over spinach purée which had no buffering agent. The addition of magnesium carbonate to the buffered spinach aided somewhat in color retention. However, when the spinach had first been buffered and subjected to conditions which promote chlorophyll to chlorophyllide conversion, the color retention after processing was much improved. This improvement was noted after storage at 100°F. (37.8°C.) in the dark for 12 weeks.

The addition of the emulsifier Triton-X-100 to spinach which contained a high proportion of chlorophyllide increased the amount of green color in the processed product, as noted by a visual panel. Even spinach which did not contain chlorophyllide after processing, but did contain this emulsifier, had a slightly greener color than without the emulsifier. This was noted after the products had been stored for 12 weeks in the dark at 100°F. (37.8°C.).

There was reason to believe that the sodium phosphate buffer improved the color of spinach not only by raising the pH of the spinach purée and increasing the amount of chlorophyll to chlorophyllide conversion, but also by tightly binding some chlorophyll and chlorophyllide to the spinach matrix. This probably prevented some of the acids, produced during the thermal process and storage period, from degrading the pigments.

When submitted to a taste panel, the sample with the magnesium carbonate and sodium phosphate buffer was preferred by a majority over the sample which had been processed with no ingredients added.

An attempt to convert chlorophyll to chlorophyllide in peas and string beans, both as purées, did not yield satisfactory results. M $3.20; X $11.25. 247 pages.

CATALYSTS OF LIPID PEROXIDATION IN MEATS

(Order No. 69-11,310) X $4.80

Hsiao-ping Liu, Ph.D.
The Florida State University, 1968

Hemoprotein and non-heme iron components are active catalysts of lipid peroxidation. The behavior of these two catalysts under a number of conditions was compared as a basis for a study of their activities in meats. In model systems, metmyoglobin accelerated linoleic acid peroxidation in a pH range from 5.6 to 7.8; it catalyzed especially rapidly at higher pH. A complex of ferrous ion and ethylene-diaminetetraacetic acid [Fe(II)-EDTA]--a non-heme iron model--in a 1:1 ratio accelerated peroxidation at lower pH; no catalysis took place above pH 6.4. Most chelating agents eliminated Fe(II)-EDTA catalysis, but had no effect on metmyoglobin catalysis. Reducing agents, on the other hand, accelerated Fe(II)-EDTA catalysis but inhibited metmyoglobin catalysis. Other sulfur compounds with -S- or -S-S- groups showed no effect on either type of catalysis.

In model systems in which fresh dilute (1.2%; W/V) meat homogenate was the catalyst, the effect of the heme predominated, i.e., the pH response was similar to that in the heme catalyzed model system and reducing agents generally inhibited peroxide formation. Ascorbic acid was an exception; it accelerated oxidation at pH 5.6. The pattern of linoleate peroxidation catalyzed by heme free (H_2O_2-treated) beef homogenate and shrimp homogenate was similar to that in the Fe(II)-EDTA model system, especially when the homogenates were studied in more dilute buffer systems where the chelating effects of the buffer did not interfere. Again, ascorbic acid accelerated the catalysis and the acceleration could be eliminated by adding chelating agents. The presence of a non-heme iron catalyst in meat tissue is thus indicated.

Evidence is presented for both types of catalytic activity in meats. In cooked meats, heme was the dominant catalyst, especially at high pH values, but significant lipid oxidation, apparently catalyzed by a non-heme iron type catalyst, occurred in cooked meats in which the heme had been destroyed by H_2O_2. In raw meats, lipid oxidation was inhibited at high pH because of removal of oxygen by enzymatic reducing systems. Both heme and non-heme iron were active at lower pH values. EDTA inhibited lipid oxidation during storage, presumably by its demonstrated effect on non-heme iron catalysis. Ascorbic acid also inhibited lipid oxidation, probably indirectly by keeping the heme pigment in the catalytic inactive ferrous state. M $3.00; X $4.80. 91 pages.

EFFECT OF TEMPERATURE DURING EARLY STAGE OF CURING UPON CHEDDAR CHEESE CHARACTERISTICS

(Order No. 69-9877) X $8.00

Md. Abdul Hamid Miah, D.V.M.S., Ph.D.
Iowa State University, 1968

Supervisor: George W. Reinbold

From a survey of 19 commercial plants, parameters for the experimental set up for studying the effect of curd cooling rates on the final quality of Cheddar cheese were established. Cheeses were manufactured on a pilot plant scale according to the experimental plan. A total of 15 lots were made. Of the 6 lots made with manufacturing-grade milk, 3 were high acid cheese with milling acidities above 0.60% and 3 were normal acid cheese with milling acidities from 0.55-0.60%. The remaining were made with grade-A milk. Of these three were made with a culture producing fruity flavor, 3 with a culture producing bitter flavor, 3 under ideal conditions, and 1 with commercial culture and a heavy inoculum of coliform organisms. Each lot of cheese was treated in four different ways with 2 pressing times (4- and 20-hr) and 2 cooling rates (rapid and slow). After 4 hr pressing, one set of wrapped and sealed blocks was immersed in brine tank at 7.5 C for rapid cooling. The other set was stacked on a pallet in a curing room held at 7.5 C for slow cooling. After 20 hr pressing cheeses were treated similarly. Periodically cheeses were tested for total count, enterococcus count, gram-negative bacterial count, lactose, glucose, galactose, pH, lactic acid, free fatty acids, and proteolysis up to 3 months. Cheeses were judged after 3 and 6 months.

Forty-pound blocks stacked in the curing room at 7.5 C took 150 to 480 hr to cool to 8 C or below. Immersing the blocks in brine at 7.5 C reduced the cooling time to 25 to 60 hr. Twenty-pound blocks required only 20 to 25 hr in brine and 135 to 185 hr in the curing room to cool to 8 C or below. No significant differences were observed in total bacterial count, enterococcus count, and gram-negative bacterial count due to cooling rates or pressing times. However, high counts of adventitious microorganisms in raw milk favored their presence in cheese during ripening.

Lactose, glucose, and galactose were found to persist in cheese beyond 90 days. Lactose content of rapidly cooled cheeses and 4-hr pressed cheeses were higher than those of air-cooled (slow) and 20-hr pressed cheeses respectively at the 1% level of significance. Brine-cooled cheeses also contained significantly higher amounts of glucose and galactose than the air cooled cheeses during all stages of ripening. The air-cooled cheeses contained significantly higher amounts of lactic acid, total free fatty acids and had low pH values during all stages of curing. No significant differences were found in the amount of proteolysis due to cooling rate or pressing time.

The air-cooled cheeses were more severely criticised for high acid, fruity, and other flavor defects. Rapid cooling tended to reduce the flavor defects in cheese. These improvements were marked in cheese made with poor quality milk. Rapid cooling did not prevent the development of bitter flavor but it improved the body and texture. Color uniformity was noticed in all brine-cooled cheeses.

M $3.00; X $8.00. 175 pages.

Institute of Therapy of Geneva University

The Effects of Inorganic Ions on the Activity of Serum Cholinesterase

II. In vivo Studies with the Guinea Pig

Ed. Frommel, A.D. Herschberg and J. Piquet

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In a preceding work (Frommel, Herschberg and Piquet [1]) we studied modifications of the activity of horse serum cholinesterase in vitro. In this way we have ascertained that a large number of inorganic ions inhibit this enzyme's activity and that others encourage enzymatic activation.

This study performed outside the living organism can only give partial results. We have judged it necessary to complete our experiments with in vivo studies, in order to be able to develop more generalised statements.

We consider these studies to be of more practical value, because in this case we can not only understand the direct toxic effect on serum cholinesterase, but also the total effect on the whole organism with its defense and equilibration faculties still intact.

We can also make more precise conclusions about the pharmacodynamics of inorganic ions and the pathogenic mechanism of certain vagotonic symptoms from intoxication by these ions.

Another point deserves to be noted in this experimental series, it is that the serums of the guinea pig and the horse do not always behave in the same way with respect to certain inhibitors. We have been able

to show recently (2) that histamine inhibits the hydrolytic capacity of guinea pig serum, while horse serum cholinesterase is not influenced by this amine, at whatever dosage used.

Is the behavior of these different types of enzymes similar toward inorganic ions? That is likewise the question which we seek to resolve.

Method

We used injections in guinea pigs, of various inorganic salts, and we studied the concomitant changes in the serum cholinesterase.

Serum cholinesterase is very variable in the guinea pig from one animal to another, but in the same individual is practically constant.

This is why we rejected the method of statistical comparison. Our guinea pigs were tested before and after the experiment. We only attach significance to the variations which were able to be verified in the same animal.

Next we established the average individual variations, in order to ascertain the total effect of the injected medicine.

We have shown in a preceding study (3) that the level of cholinesterase in the guinea pig is subject to seasonal variations: This level is high in the spring and in summer, and low in winter. We attributed this variation to a deficiency in winter in the Vitamin C content of their food. It also appears that cold likewise plays a part. For this reason the animals were kept at constant temperature and received an identical diet. Our experiments only lasted a maximum of 8-10 days for each series, an insufficient period to bring about a variation in cholinesterase level due to external circumstances.

Blood samples were taken by a cardiac tap. They rarely exceeded 1-15 cm of blood and were spaced apart at least 48 hours. We know in fact that blood letting lowers the cholinesterase level, but recuperation takes place in less than 48 hours. The blood is allowed to coagulate at ambiant temperature and waits for the clot to shrink before putting the blood in the refrigerator. The determination was never made much later than 24 hours after taking a sample.

The determination was carried out, as with the horse serum, by the Hall and Lucas method (4).

The injected solutions were administered subcutaneously and rarely intramuscularly. We never used the intravenous route.

The injection of some drugs gave rise to small cutaneous scabs, incapable of altering the serum cholinesterase level.

Results

In order to avoid repetition, we give our experimental results in the form of a synopsis table that we discuss afterward.

As one can see from the table, the general reactions of serum cholinesterase in the guinea pig take place in the same way as in our in vitro experiments on horse serum.

On the other hand, one sees a definite quantitative difference. These differences furthermore can be explained by rather simple facts. A number of organs are involved in the regulation of serum cholinesterase. The most important of these organs is the liver. Certain authors [Antopol, 5; Butt, 6; Hawes and Alles, 7; McArdle, 8; Vincent, 9] in fact have shown that in cirrhosis of the liver, hepatitis and jaundice, the activity of cholinesterase in the serum was lowered.

In addition, thyroid deficiency (Antopol, Tuchman and Shifrin, 10; Werle and Stuttgen, 11), likewise entails a slowing down of enzymatic hydrolysis of acetylcholine.

Beyond doubt, in the toxicity by inorganic ions that we have produced in the guinea pig, we have strongly disturbed the various functions of the glandular parenchyme and have created anatomical problems.

To the direct action of the ion on cholinesterase, which we have established in vitro, is now added the modification of this enzyme from the organic injury of the viscera.

On the other hand, in other cases, the living organism reacts by neutralising the toxic action of certain ions by a mechanism that we scarcely understand. One could postulate a neoformation of enzyme having the reestablishment of the initial enzyme level as its purpose.

Finally we should mention some particular cases.

1. Divergences with in vitro experiments

NaBr. If we compare the results obtained in the in vitro experiment on horse serum with the in vivo studies in the guinea pig, we find a definite difference. NaBr is in fact a strong inhibitor of cholinesterase in horse serum in vitro, while it accelerates that of the guinea pig in vivo.

It is the same for the double tartrate of Sb and Na. A slight inhibitor in vitro, it is practically inactive on the serum cholinesterase of the guinea pig in vivo.

An inverse action is found with $CaCl_2$ which is an activator in vitro and is an inhibitor in vivo. The same for calcium hyposulfite.

An analogous discrepancy is seen with injections of K_2SO_4 a slight inhibitor in vitro, inactive in vivo.

Table 1

Ions	Guinea pig	Weight	*Dose received in g/kg	CHE before	CHE after	Result in %	Average
Sodium hyposulfite	f	480	5 x 0,50	2,77	3,45	+24%	+41%
	m	430	5 x 0,50	1,84	2,93	+58%	
Magnesium chloride	m	410	4 x 1,0	2,40	3,60	+33%	+34.5%
	m	430	5 x 1 g	2,05	2,75	+34%	
	m	570	2 x 1 g	1,40	1,90	+36%	
Manganese chloride	m	505	4 x 0,01	2,01	1,10	-45%	-37%
	m	400	4 x 0,01	1,55	1,10	-29%	
Aluminum sulfate	m	540	2 x 0,01	2,45	2,44		
	m	480	3 x 0,01	2,60	2,05	-21%	
	m	500	4 x 0,01	2,93	2,51	-15%	

*The injected dose was calculated according to Aberhalden (Handbook of Biological Work Methods 1, 7) to obtain the chronic toxicity for the ion studied.

Similarly manganese chloride does not seem to be active in vitro on horse serum, but in vivo inhibits cholinesterase in guinea pig serum.

These various discrepancies are interesting. It is possible that introducing certain ions in a living organism releases a number of compensating mechanisms. One of the mechanisms which can be invoked in the case of injections of Ca salts, is a liberation of K ions, which is a cholinesterase inhibitor. Does this reaction exceed its purpose? That is what we allow ourselves to surmise.

The inverse is produced in the case of injections of K salts.

But another hypothesis should be examined: our experiments in vitro were carried out on the cholinesterase of horse serum; those in vivo, on the cholinesterase of guinea pig serum. But a number of previous studies showed different cholinesterase reactions in various animal species. In particular, there certainly exists a difference in behavior between horse serum cholinesterase and that of the guinea pig, the more so because the horse, contrary to the guinea pig, possesses a strong vagus tonus.

Consequently, we have redone the study of the action of ions on guinea pig serum. Guinea pig serum being extremely difficult to obtain in a sufficient amount, we limited this verification to only two ions, Br and Sb, because if we can accept equilibrium mechanisms for normal ions present in serum (Ca, K), it is more difficult to picture analogous reactions for ions foreign to serum (at least in considerable amounts).

The study of cholinesterase in guinea pig serum gave us the following results.

Bromide and sodium exert a pronounced inhibition on guinea pig cholinesterase, just as that with horse serum. The difference found between

the in vivo and in vitro studies does not reside therefore in a different reactivity of the cholinesterases of dissimilar species, but must be sought in a compensatory mechanism which occurs at the moment the bromide is introduced into the living organism.

As for manganese chloride, neutral with the cholinesterase of horse serum and inhibiting when injected in the guinea pig, it induces a positive acceleration of cholinesterase when put in direct contact with guinea pig serum.

Here, besides the compensatory phenomenon, it is possible that the difference in the origin of the serums comes into play.

These facts limit still further the general statements that we have made in this study, in the sense that the results obtained cannot be extended to other species than those which we have studied.

2. Experiments in reactivation. Nachmansohn and Lederer have pointed out what we have already said repeatedly, that Ca^{2+} ion reactivates cholinesterase.

Seeking to determine the mechanism of the desensitization action of some compounds used, for therapeutic purposes, we have been able to show that ascorbic acid, for example (Frommel, Herschberg and Piquet, 3), inactive with whole cholinesterase, returns its activity to cholinesterase inhibited by various toxins. It is the same for Ca. We have been able to show (15, d) that if one lowers the activity of guinea pig serum cholinesterase by injections of Myosalvarsan (0.08 g/kg) one gets an enzyme fall of about 70%. If one treats this guinea pig with simultaneous injections of calcium gluconate (0.40 g/kg) this lowering is only 35% on the average.

Calcium is therefore a reactivator of cholinesterase in vivo, while at the same time in vitro, it returns its activity to cholinesterase destroyed by dialysis.

Additionally, one sees that a cholinesterase relay exists in the desensitization effect of calcium.

3. Compounds studied uniquely in vivo or in vitro. Our experimentation on the guinea pig was carried out with a certain number of ions not studied in vitro. The reason for this is that one can inject oily solutions in the animal (phosphoric oil, bismuth oleate) which does not lend itself to in vitro studies, which require colorless, aqueous solution. Contrarily, some ions cannot be injected in the guinea pig because of their great causticity, such as silver nitrate, iron sulfate, etc. For all these compounds we were obliged to settle for in vitro experiments.

Conclusions

The experiments for which we have just given detailed results have apart from their purely theoretical interest, a practical interest, even clinical in the sense that they appear to be able to give us the key to the pathology of the symptoms of certain intoxications.

It is curious, for example, to show by analysing the manifestations of lead poisoning, the major part of the clinical disturbances are the consequences of a strong hypervagotonia (colic, spasms of the smooth musculature, muscular problems). Now we have just shown that Pb is one of the strongest inhibitors of cholinesterase, both in vivo and in vitro. It is easy to imagine that the symptoms of vagotonia could appear when acetylcholine is no longer sufficiently destroyed by an inhibited cholinesterase.

Moreover, hasn't clinical therapy anticipated our experimental demonstration by using atropine or adrenaline against lead poisoning symptoms?

It is the same with antimony poisoning. The animal poisoned with massive doses of Sb shows copious salivation, nausea, vomiting, diarrhea, asthmatic dysprea, and finally muscular asthenia terminating in death with violent convulsions.

Paralleling this clinical picture, the heart is strongly depressed and the pressure falls. The heart ceases by stopping in diastole.

But, this syndrome, which is that of an acute hypervagotonia, point by point agrees with that produced by a massive intravenous injection of acetylcholine.

On the other hand, if one takes the group of drugs called "desensitizers" which are the hyposulfites of Na, Ca or Mg and the salts of Ca or Mg, we see that the entire group are cholinesterase activators as much in vitro as in vivo. It is that finding which seems interesting to us, and which contributes to the pharmacodynamics of these ionic mineral medicines.

We recall that these activating ions, all of which have a considerable effect on intact cholinesterase, exert a powerful effect above all on cholinesterase which has been partially destroyed by a poison. We developed these ideas in a preceding study (12).

In that way, we came upon ascorbic acid another cholinesterase reactivator. We showed in fact that ascorbic acid, probably a coenzyme of cholinesterase, does not act on intact cholinesterase in vivo or in vitro, but restores its former activity to previously inhibited cholinesterase.

It is in this way that we have been able to show that Ca^{2+} counteracts the inhibition of cholinesterase produced by neoarsphenamine. The protective action of ascorbic acid against arspehamine symptoms can be similarly interpreted.

Summary

Having established in a previous study that horse serum cholinesterase is subject to functional modifications under the influence of certain inorganic ions, we attempted to study the effect of these ions on cholinesterase in vivo.

To this end, we injected guinea pigs with inorganic salts, and we measured the enzymatic activity of the serum (drawn off by successive cardiac punctures), by the titrimetric method of Hall and Lucas, before and after the injections.

Most of the ions studied inhibited the serum cholinesterase of the guinea pig. The inhibition varied according to the ion or the reactions of each individual.

Other ions were activators of intact cholinesterase, some finally restored the activity of a previously inhibited enzyme.

In general, the results obtained paralleled those of the first experimental series on horse serum in vitro.

On the other hand, we have found some discrepancies which we have attempted to interpret either as a difference in the origin of the serum or by a compensatory phenomenon produced in a living organism.

The practical interest in this group of experiments is to contribute some explanations for the pathogenesis of certain vagotonic symptoms of acute or chronic poisoning from the therapeutic use of various inorganic ions.

Helv. Physiol. Acta 2, 193–201 (1944)

Institut de Thérapeutique de l'Université de Genève

Effets des ions inorganiques sur l'activité de la cholinestérase sérique

II. Études, in vivo, sur le cobaye

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Dans un précédent travail (*Frommel, Herschberg* et *Piquet* [1]) nous avons étudié les modifications de l'activité de la cholinestérase du sérum de cheval in vitro. Nous avons ainsi pu constater qu'un grand nombre d'ions inorganiques inhibent l'action de ce ferment et que d'autres ions entrainent une activation enzymatique.

Cette étude faite hors de l'organisme vivant ne pouvait que donner des résultats partiels. Nous avons jugé nécessaire, afin de pouvoir tendre vers des constatations plus générales, de compléter nos expériences par des essais in vivo.

Nous attribuons à ces essais une plus grande valeur pratique qu'à la première série, car dans ce cas nous pouvons connaître non seulement l'effet direct du toxique sur la cholinestérase sérique, mais encore l'effet global de ce toxique sur un organisme conservant intactes toutes ses facultés de défense et d'équilibration.

Nous pouvons également tirer des conclusions plus précises sur la pharmacodynamie des ions inorganiques et le mécanisme pathogénique de certains symptomes vagotoniques des intoxications par ces ions.

Un autre point mérite d'être signalé dans cette série expérimentale, c'est que les sérums de cobaye et de cheval ne se comportent pas toujours de la même manière vis-à-vis de certains inhibiteurs. Nous avons pu montrer récemment (2) que l'histamine inhibe le pouvoir hydrolytique du sérum de cobaye, alors que la cholinestérase sérique du cheval n'est pas influencée par cette amine, quelles qu'en soient les doses utilisées. Le comportement de ces enzymes d'espèces différentes est-il identique envers les ions inorganiques? C'est également la question que nous avons cherché à résoudre.

Méthode

Nous avons pratiqué des injections de divers sels inorganiques à des cobayes et nous avons étudié les variations concomitantes de la cholinestérase sérique.

La cholinestérase du sérum de cobaye est très variable d'un animal à l'autre, mais est pratiquement constante chez un même individu.

C'est pourquoi, nous rejetons la méthode de travail par comparaison statistique. Nos cobayes ont été testés avant et après l'expérience; nous n'attachons d'importance qu'aux variations pouvant être constatées chez un même animal.

Puis nous avons établi les moyennes des variations individuelles, afin de constater l'action globale du médicament injecté.

Nous avons montré dans un travail précédent (3) que le taux de la cholinestérase chez le cobaye subit des variations saisonnières: ce taux est haut au printemps et en été, il est bas en hiver. Nous avons attribué cette variation à une déficience hivernale en vitamine C alimentaire. Il semble que le froid joue également un certain rôle. Ce sont les raisons pour lesquelles les animaux furent gardés à une température constante et reçurent une nourriture identique; nos expériences ne durèrent pour chaque série qu'un maximum de 8-10 jours, période insuffisante pour entraîner une variation du taux cholinestérasique due à des circonstances extérieures.

Les prises de sang furent faites par ponctions cardiaques. Elles ne dépassaient que rarement 1–1,5 ccm de sang et étaient espacées d'au moins 48 heures. Nous savons en effet que la saignée baisse le taux de la cholinestérase sérique, mais que la récupération se fait en moins de 24 heures. On laisse le sang se coaguler à la température ambiante et on attend que le caillot se rétracte avant de mettre le sang à la glacière. Le dosage a jamais été fait plus tard que 24 heures après son prélèvement.

Le dosage fut pratiqué, comme pour le sérum de cheval, par la méthode de *Hall* et *Lucas* (4).

Les solutions injectées furent administrées par voie sous-cutanée, rarement par voie intramusculaire. Nous n'avons jamais utilisé la voie intra-veineuse.

L'injection de certaines drogues a donné lieu à de petites eschares cutanées, incapables de modifier le taux de la cholinestérase sérique.

Résultats

Afin d'éviter les répétitions, nous donnons nos résultats expérimentaux sous forme d'un tableau synoptique, que nous commenterons ensuite.

Ions	Cobaye	Poids	Dose reçue*) en g/kg	CHE avant	CHE après	Résultat en %	Moyenne
			Anions				
Bromure de sodium .	m	340	5 × 0,50	2,30	2,67	+12%	+17%
	m	355	5 × 0,50	2,50	3,10	+24%	
	m	480	5 × 0,50	2,36	2,70	+14%	
Iodure de sodium . .	m	540	4 × 0,20	2,14	1,52	−29%	−28%
	m	525	4 × 0,20	2,16	1,58	−27%	
Hyposulfite de sodium	f	480	5 × 0,50	2,77	3,45	+24%	+41%
	m	430	5 × 0,50	1,84	2,93	+58%	

*) Les doses injectées furent calculées d'après *Abderhalden* (Handbuch der biologischen Arbeitsmethoden, 7, 7), pour obtenir une intoxication chronique par l'ion étudié.

Ions	Cobaye	Poids	Dose reçue*) en g/kg	CHE avant	CHE après	Résultat en %	Moyenne
Myosalvarsan[1]) . . .	m	520	4 × 0,04	2,95	1,30	−56%	−55%
	m	530	4 × 0,04	3,44	1,60	−54%	
	m	520	6 × 0,04	3,15	0,84	−77%	−69%
	m	560	6 × 0,04	3,40	1,27	−61%	
Tartrate double de Sb et K	m	590	3 × 0,003	1,85	1,30	−30%	−16%
	m	480	3 × 0,003	1,90	1,85	− 2%	
Tartrate double de Sb et Na	m	640	6 × 0,02	1,04	1,10	+ 3%	+ 1%
	m	460	6 × 0,02	1,05	1,03	− 1%	
Huile phosphorée[2]) .	f	610	2 × 1 ccm	2,58	1,59	−39,5%	−46%
	f	550	2 × 1 ccm	2,85	1,38	−52%	
Borate de sodium . .	m	360	2 × 0,30	3,99	3,50	−12,5%	
	m	420	3 × 0,30	3,62	2,39	−34%	
	m	380	4 × 0,30	3,95	2,75	−31%	
			Cations				
Chlorure d'ammonium[3])	m	480	4 × 0,05	1,85	2,33	+26%	+18%
	m	340	4 × 0,05	2,56	2,82	+10%	
Sulfate de lithium . .	m	610	4 × 0,05	1,60	1,33	−17%	−17%
	m	680	4 × 0,05	1,43	1,20	−17%	
Sulfate de potassium	m	350	2 × 0,30	2,93	2,80	− 5%	
	f	430	3 × 0,30	3,00	3,10	+ 3%	
	f	380	4 × 0,030	2,60	2,74	+ 5%	
Hyposulfite de Calcium	m	580	3 × 0,20	0,87	0,86	0	− 8%
	m	540	3 × 0,20	1,28	1,08	−15%	
	m	400	3 × 0,20	1,20	1,05	−10%	
Galactogluconate de Calcium	f	540	5 × 0,40	3,10	3,27	+ 5%	+ 5%
	f	510	5 × 0,40	2,70	2,87	+ 5%	
Chlorure de Calcium .	m	480	4 × 0,10	2,57	1,65	−36%	−35,5%
	m	535	4 × 0,10	1,89	1,24	−35%	

*) Les doses injectées furent calculées d'après *Abderhalden* (Handbuch der biologischen Arbeitsmethoden, 7, 7), pour obtenir une intoxication chronique par l'ion étudié.

[1]) Le Myosalvarsan est une variante du Novarsénobenzol, injectable par voie intramusculaire.

[2]) L'huile phosphorée du Codex, que nous avons employée, est une huile de vaseline fluide contenant, en solution, 0,475–0,525% de phosphore.

[3]) Le pH du sérum de cobayes traités par les injections de chlorure d'ammonium ne différait pas de celui d'animaux témoins.

Ions	Cobaye	Poids	Dose reçue*) en g/kg	CHE avant	CHE après	Résultat en %	Moyenne
Chlorure de Magnesium	m	410	4 × 1,0	2,40	3,60	+33%	
	m	430	5 × 1 g	2,05	2,75	+34%	+34,5%
	m	570	2 × 1 g	1,40	1,90	+36%	
Hyposulfite de magnesium	m	410	3 × 1,0	1,94	1,91	— 1%	
	m	640	3 × 1,0	1,66	1,85	+11%	+ 5%
Chlorure de baryum	m	380	4 × 0,01	2,37	1,70	—29%	
	m	440	3 × 0,01	1,82	1,71	— 9%	—19%
Sulfate de zinc	f	425	2 × 0,075	3,80	3,51	— 8%	
	f	350	3 × 0,075	3,55	2,54	—19%	
	f	520	4 × 0,07	3,35	1,79	—46%	
Chlorure de manganèse	m	505	4 × 0,01	2,01	1,10	—45%	
	m	400	4 × 0,01	1,55	1,10	—29%	—37%
Acetate de nickel	m	230	2 × 0,01	2,93	2,68	— 8%	
	m	400	3 × 0,01	2,90	2,95	+ 2%	
	m	350	4 × 0,01	4,93	3,00	+ 2%	
Chlorure stanneux	f	430	2 × 0,01	3,62	3,05	—16%	
	f	500	3 × 0,01	3,30	2,75	—18%	
	f	380	4 × 0,01	4,00	1,99	—50%	
Sulfate d'aluminium	m	540	2 × 0,01	2,45	2,44		
	m	480	3 × 0,01	2,60	2,05	—21%	
	m	500	4 × 0,01	2,93	2,51	—15%	
Protéinate d'argent	f	560	2 × 0,05	1,86	1,03	—45%	
	f	340	3 × 0,05	3,77	2,30	—39%	
	f	610	4 × 0,05	3,80	1,59	—59%	
Myoral[4])	m	565	5 × 0,015	4,12	2,06	—50%	
	m	575	5 × 0,015	3,22	2,69	—16%	—40%
	m	550	5 × 0,015	4,30	2,06	—55%	
Acetate de Pb-triéthyle	f	720	2 × 0,01	3,37	1,50	—56%	
	f	560	2 × 0,01	2,80	1,35	—52%	—49%
	f	440	2 × 0,01	3,20	2,00	—38%	
Sulfate de cuivre	m	480	4 × 0,01	1,42	0,95	—31%	
	m	370	4 × 0,01	1,36	1,28	— 6%	—19%

*) Les doses injectées furent calculées d'après *Abderhalden* (Handbuch der biologischen Arbeitsmethoden, **7**, 7), pour obtenir une intoxication chronique par l'ion étudié.

[4]) Le Myoral est un composé aurique en solution huileuse injectable par voie intramusculaire. Sa formule chimique est: aurothioglycolate de Ca, et sa teneur en Au: 64,16%.

Ions	Cobaye	Poids	Dose reçue*) en g/kg	CHE avant	CHE après	Résultat en %	Moyenne
Sublimé	f	590	2 × 0,02	2,20	1,83	—17%	
	f	590	2 × 0,02	3,44	0,88	—75%	—46%
	m	630	3 × 0,02	2,33	mort		
	m	530	3 × 0,02	2,20	mort		
Merfen[5])	m	470	3 × 1 ccm	1,24	0,74	—41%	
	m	560	3 × 1 ccm	1,79	1,40	—22,5%	—32%
Oléo-Bi[6])	f	600	5 × 1 ccm	2,94	1,48	—55%	
	f	680	8 × 1 ccm	2,58	1,70	—35%	—45%
Ésérine	m	350	2 × 0,0005	3,55	0,85	—76%	
	m	420	3 × 0,0005	3,84	1,48	—62%	—68%

*) Les doses injectées furent calculées d'après *Abderhalden* (Handbuch der biologischen Arbeitsmethoden, **7**, 7), pour obtenir une intoxication chronique par l'ion étudié.

[5]) Le «Merfen» est un produit désinfectant du commerce, dont la formule est: borate de phényl-hydrargyre. Nous avons utilisé ce produit afin de comparer l'action d'un Hg organique avec celle d'un Hg minéral (sublimé). Sol. aq. à 2‰.

[6]) L'oléo-Bi est un oléate de bismuth, titrant 0,012 g par ccm.

Discussion

Comme on peut le voir sur le tableau, les réactions générales de la cholinestérase sérique du cobaye se font dans le même sens que dans nos expériences in vitro sur le sérum de cheval.

Par contre l'on assiste à de nettes *différences quantitatives*. Ces différences quantitatives peuvent d'ailleurs être expliquées par des faits assez simples. Nombre d'organes interviennent dans la régulation de la cholinestérase sérique. Le plus important de ces organes est le foie. Certains auteurs (*Antopol* [5], *Butt* [6], *Hawes* et *Alles* [7], *McArdle* [8], *Vincent* [9]) ont en effet constaté que dans les cirrhoses du foie, les hépatites et les ictères, il y avait une baisse d'activité cholinestérasique dans le sérum.

Par ailleurs, les insuffisances thyroïdiennes (*Antopol*, *Tachman* et *Shifrin* [10], *Werle* et *Stättgen* [11]), entraînent également un ralentissement de l'hydrolyse enzymatique de l'acétylcholine.

Dans l'intoxication par ions inorganiques que nous avons produite chez le cobaye, il est hors de doute que nous avons perturbé fortement le fonctionnement des divers parenchymes glandulaires et que nous avons créé des troubles anatomiques.

A l'action directe du ion sur la cholinestérase, que nous avons constatée in vitro, vient s'ajouter donc la modification de ce ferment par lésion organique des viscères.

Par contre, l'organisme vivant réagit, dans d'autres cas, en neutralisant par des mécanismes que nous ne connaissons que mal l'action toxique de certains ions. On pourrait supposer une néo-formation de ferment, ayant pour but de rétablir le taux enzymatique initial.

Il nous faut enfin mentionner quelques cas particuliers:

1. Divergences avec les expériences in vitro

BrNa. Si nous comparons les résultats obtenus dans l'expérience in vitro sur le sérum de cheval avec ceux des essais in vivo sur cobayes, nous constatons une nette différence. En effet, le BrNa est un inhibiteur puissant de la cholinestérase du sérum de cheval in vitro, alors qu'il accélère celle du cobaye in vivo.

Il en est de même pour le *tartrate double de Sb et Na*. Léger inhibiteur in vitro, il est pratiquement inactif sur la cholinestérase du sérum de cobaye in vivo.

Une action inverse peut être constatée avec le Cl_2Ca, qui, activateur in vitro, est inhibiteur in vivo. Idem pour l'*hyposulfite de Ca*.

Une discordance analogue se révèle avec les injections de SO_4K_2, inhibiteur légèr in vitro, inactif in vivo.

De même, le *chlorure manganeux* ne paraît pas avoir d'action in vitro sur le sérum de cheval, mais inhibe, in vivo, la cholinestérase du sérum de cobaye.

Ces quelques divergences sont intéressantes. Il est possible que l'introduction de certains ions dans un organisme vivant déclenche de nombreux mécanismes compensateurs. Un de ces mécanismes, que l'on peut invoquer dans le cas des injections de sels de Ca, est une libération de ions K, qui est un inhibiteur de la cholinestérase. La réaction dépasse-t-elle le but? C'est ce qu'il nous est permis de supposer.

L'inverse se produirait en cas d'injections de sels de K.

Mais une autre hypothèse doit être examinée: nos expériences in vitro portent sur une cholinestérase de sérum de cheval; celles in vivo, sur une cholinestérase de sérum de cobaye. Or, de nombreux travaux antérieurs montrent les différentes réactions des cholinestérases de diverses espèces animales. En particulier, il existe certainement une différence de comportement entre les cholinestérases sériques de cheval et de cobaye, d'autant plus que le cheval, contrairement au cobaye, possède un fort tonus du vague.

Nous avons par conséquent refait l'étude de l'action ionique sur le sérum de cobaye in vitro. Le sérum de cobaye étant fort difficile à obtenir en quantité suffisante, nous avons dû limiter cette vérification à deux ions seulement: le Br et le Sb, car si nous pouvons admettre des mécanismes équilibrateurs pour des ions normalement présents dans le sérum (Ca, K), il est plus difficile de s'imaginer des réactions analogues pour des ions étrangers au sérum (du moins en quantité notable).

L'étude de la cholinestérase du sérum de cobaye nous a donné les résultats suivants:

Le *bromure de sodium* exerce une inhibition prononcée sur la cholinestérase du sérum de cobaye, tout comme sur celle du sérum de cheval. La différence constatée entre les essais in vivo et in vitro ne réside donc pas en une différente réactivité des cholinestérases d'espèces éloignées, mais doit être recherchée dans un mécanisme compensateur qui se produirait lors de l'introduction de bromure dans un organisme vivant.

Quant au *chlorure de manganèse*, indifférent sur la cholinestérase du sérum de cheval et inhibiteur en injection au cobaye, il provoque une certaine accélération de la cholinestérase quand il est mis en contact direct avec le sérum de cobaye.

Ici, outre les phénomènes compensateurs, il est possible que la différence d'origine des sérums entre en jeu.

Ces faits limitent encore davantage les constatations générales que nous avons faites dans ce travail, dans le sens que les résultats obtenus ne peuvent être étendus sans autre aux espèces autres que celles que nous avons étudiées.

2. Expériences de réactivation

Nachmansohn et *Lederer* ont indiqué, comme nous l'avons déjà dit à plusieurs reprises, que l'ion Ca^{++} est un réactivateur de la cholinestérase.

Cherchant à déterminer le mécanisme de l'action «désensibilisante» de certains corps utilisés dans ce but en thérapeutique, nous avons pu montrer que l'acide ascorbique par exemple (*Frommel, Herschberg* et *Piquet* [3]), inactif sur une cholinestérase intacte, rendait son activité à une cholinestérase inhibée par divers toxiques. Il en est de même pour le Ca. Nous avons pu constater (15, d) que si l'on baisse l'activité de la cholinestérase sérique du cobaye par des injections de Myosalvarsan (0,08 g/kg) on obtient une chute fermentaire de 70% environ. Si l'on traite ces cobayes par des injections simultanées de gluconate de calcium (0,40 g/kg) cette baisse n'est plus que de 35% en moyenne.

Le calcium est donc un réactivateur de la cholinestérase in vivo, tout comme il rend, in vitro, son activité à une cholinestérase détruite par dialyse.

Accessoirement, on voit qu'il existe un relai cholinestérasique dans l'action désensibilisante du calcium.

3. Corps étudiés uniquement in vivo ou in vitro

Notre expérimentation sur cobayes porte sur un certain nombre d'ions non étudiés in vitro. La raison de ce fait est que chez l'animal on peut injecter des solutions huileuses (huile phosphorée, oléate de bismuth) qui ne se prêtent pas à l'étude in vitro, qui nécessitent des solutions aqueuses incolores. Inversément, certains ions n'ont pas pu être injectés aux cobayes

à cause de leur grande causticité, comme le nitrate d'argent, le sulfate de fer, etc. Pour tous ces corps, nous avons dû nous contenter des expériences in vitro.

Conclusions

Les expériences dont nous venons de donner les résultats détaillés ont, en dehors de l'intérêt purement théorique, un intérêt pratique et même clinique dans le sens qu'elles semblent devoir nous donner la clé de la pathogénie des symptômes de certaines intoxications.

Il est curieux, par exemple, de constater en analysant les manifestations de l'intoxication saturnine, que la majeure partie des troubles cliniques sont des conséquences d'une forte hypervagotonie (coliques, spasmes de la musculature lisse, troubles musculaires). Or, nous venons de montrer que le Pb est un des inhibiteurs les plus puissants de la cholinestérase, tant in vivo qu'in vitro. On imagine facilement que les symptômes vagotoniques puissent apparaître, lorsque l'acétylcholine n'est plus suffisamment détruite par une cholinestérase inhibée.

D'ailleurs, la thérapeutique clinique n'a-t-elle pas devancé notre démonstration expérimentale en utilisant contre les accidents saturnins l'atropine ou l'adrénaline?

Il en est de même dans l'intoxication stibiée: l'animal intoxiqué par des doses massives de Sb présente une salivation abondante, des nausées, des vomissements, de la diarrhée, une dyspnée asthmatiforme, enfin une asthénie musculaire aboutissant à la mort dans de violentes convulsions.

Parallèlement à ce tableau clinique, le cœur est fortement déprimé, la tension tombe. Le cœur finit par s'arrêter en diastole.

Or, ce syndrome, celui d'une hypervagotonie aiguë, se superpose point par point à celui produit par une injection massive intra-veineuse d'acétylcholine.

D'autre part, si l'on prend la série des médicaments dits «désensibilisants» qui sont l'hyposulfite de Na, de Ca ou de Mg, les sels de Ca ou de Mg, on voit que toute la série est activatrice de la cholinestérase tant in vitro qu'in vivo. C'est là une donnée qui nous paraît intéressante et qui contribue à la pharmacodynamie de ces médicaments ioniques minéraux.

Nous rappelons que ces ions activateurs, tout en ayant une action notable sur la cholinestérase intacte, exercent une action puissante surtout sur une cholinestérase partiellement démolie par un toxique. Nous avons développé ces notions dans un travail précédent (12).

Par là, ils s'approchent d'un autre réactivateur de la cholinestérase, l'acide ascorbique. Nous avons en effet montré que l'acide ascorbique, co-ferment probable de la cholinestérase, n'agit pas sur la cholinestérase intacte, in vivo et in vitro, mais rend l'activité première à une cholinestérase préalablement inhibée.

C'est ainsi que nous avons pu montrer que le Ca^{++} s'oppose à l'inhibition de la cholinestérase produite par le Novarsénobenzol. L'action protectrice de l'acide ascorbique contre les accidents salvarsaniques peut être interprétée de la même manière.

Résumé

Ayant constaté, dans un précédent travail, que la cholinestérase sérique du cheval subit des modifications fonctionnelles sous l'influence de certains ions inorganiques, nous avons cherché à étudier l'effet de ces ions sur une cholinestérase in vivo.

A cette fin, nous avons injecté des sels inorganiques à des cobayes, et nous avons mesuré l'activité enzymatique du sérum (prélevé par ponctions cardiaques successives), par la méthode titrimétrique de *Hall* et *Lucas*, avant et après les injections.

La plupart des ions étudiés donnent une inhibition de la cholinestérase sérique du cobaye, inhibition variable suivant les ions et suivant les réactions de chaque individu.

D'autres ions sont activateurs de la cholinestérase intacte, quelques uns enfin rétablissent l'activité d'un ferment préalablement inhibé.

En général, les résultats obtenus sont parallèles à ceux de la première série d'expériences, sur le sérum de cheval in vitro.

Par contre nous avons constaté certaines divergences que nous nous sommes efforcés d'interpréter, soit par la différence d'origine des sérums, soit par des phénomènes compensateurs se produisant dans un organisme vivant.

L'intérêt pratique de cette série expérimentale est d'apporter quelques éclaircissements à la pathogénie de certains symptomes vagotoniques des intoxications aigues ou chroniques par divers ions inorganiques employés en thérapeutique.

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Determination of Calcium and Magnesium in Serum, Urine, Diet, and Stool by Atomic Absorption Spectrophotometry

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Atomic absorption spectrophotometry was evaluated as a method for the determination of calcium and magnesium in serum, urine, diet, and stool, and was found a most suitable technic for a large clinical chemistry laboratory.

ATOMIC ABSORPTION was developed by Walsh and his associates (*1*) as an analytical technic applicable to the determination of many metals. Willis (*2–4*) found that this technic was applicable in particular to the determination of calcium and magnesium in serum and urine. These published results indicated that atomic absorption spectrophotometry would be a simple, rapid, and practical method for the determination of calcium and magnesium in biologic material, particularly in serum and urine, and that it could be adapted effectively to the needs of a clinical chemistry laboratory. After evaluation, we have used atomic absorption spectrophotometry for routine determination of calcium and magnesium in serum and urine since November 1962.

Since our studies of this technic began, several reports have appeared on the determination of calcium and magnesium in biologic materials (*5–12*), indicating considerable variation in the methods used. Thus, it seems appropriate to publish the procedures we have found useful for routine determinations of these elements, not only in serum and urine, but also in diet and stool.

Instrumentation

The instrument used in this work, and still in routine operation, is the Perkin-Elmer Model 214 atomic absorption spectrophotometer (*13*),

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Some of the data on the determination of serum magnesium are from a dissertation in 1962 by the senior author in partial fulfillment of the requirements for the degree of Master of Arts, Hunter College, New York.

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connected to a Sargent Model SR recorder. All data recorded were collected with the burner described by Slavin (*14*). A lean acetylene-air flame has been used for all analyses. The air compressor used supplies oil-free air. Both calcium and magnesium are determined with the use of hollow cathode lamps. The 4226-A calcium line and the 2852-A magnesium line are isolated with the monochromator of the instrument set to a spectral slit width of about 300 μ. The settings of the instrument for the burner, the hollow cathode lamps, and the external recorder depend upon the particular components used. Some details on these components with respect to the determination of calcium are reported by Slavin *et al.* (*15*).

In general, the difficulties encountered with the instrument, including the burner, have been no greater than those an experienced laboratory worker might expect with any laboratory instrument or apparatus of equal complexity. Operation of the instrument has been largely trouble-free.

The slot of the burner, which is 0.020 in., tends to become constricted slightly when sera are analyzed. Currently, 27 ± 9 sera are analyzed for calcium and roughly 2 ± 2 for magnesium each day the instrument is in use. Under these operating conditions and with the diluents described, the burner is cleaned once a day. How many more sera could be analyzed without the burner's requiring cleaning remains to be determined.

Standards and Samples

Calcium carbonate (Mallinckrodt's Primary Standard) dissolved in concentrated HCl served as the stock standard for calcium. This stock solution was diluted appropriately to obtain the working standards, made up to contain the equivalent of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mg./100 ml. in a 1:10 dilution of serum.

Magnesium turnings (Mallinckrodt) dissolved in concentrated HCl served as the stock standard for magnesium. This stock solution was diluted to give working standards equivalent to 0.5, 1.0, 2.0, 3.0, and 4.0 mg./100 ml. magnesium in a 1:25 dilution of serum.

Methods and Results

Calcium

In Serum

To measure the accuracy with which calcium could be determined in serum and urine by atomic absorption spectroscopy, both comparison and recovery studies were carried out. For serum calcium, Willis (*2*) deproteinized the serum with trichloroacetic acid. He also reported that

reasonably accurate results could be achieved when serum was simply diluted with either water or 1% (w/v) EDTA. To achieve maximum accuracy with only water as the diluent, he found careful flame adjustment necessary. Since we were seeking the simplest procedure, requiring the least manipulation and the fewest steps, we used 1% EDTA as the diluent.

To determine serum calcium, 0.5 ml. of serum is diluted with 4.5 ml. of 1% EDTA. With this diluent, 100.1% of calcium added to serum (5 samples) was recovered. The coefficient of variation (V) was 1.6%; the standard deviation of the sample(s) was 1.62; and s^2 was 2.62. These values compare favorably with the 100.1% recovery reported by Willis, with V of 1.9% (calculated by us).

To test the relative accuracy of the method further, serum calcium was determined on sera which had been submitted for routine analysis and which constituted a representative sample of abnormal sera; values obtained by atomic absorption were compared with those obtained by oxalate precipitation and permanganate titration as described by Clark-Collip (*16*) (a modification of the Kramer-Tisdall method (*17*) for the chemical determination of calcium). If both methods measure serum calcium with the same accuracy, the value obtained by atomic absorption spectrophotometry (y) should equal the value obtained by the oxalate-permanganate method (x). With perfect correlation the best straight line to fit the data would have a slope of 1.0 and an intercept of 0.

A series of 56 determinations was run by both methods, on 10 different days. The number of determinations per day was 5 or fewer on 7 days, and 8, 9, and 15, respectively, the remaining 3 days.

To reduce the amount of calculation, 28 of the parallel sets of determinations (or every other one, beginning with the first), were used for statistical evaluation. The straight line which best fit these data is described by the equation $y = 0.55 + 0.95\ x$, where y is the value obtained by atomic absorption for serum calcium and x the value by the oxalate-permanganate procedure. The deviation of the slope from 1.0 and the intercept from 0 is not significant, since t_a is equal to 1.565 and t_b is equal to 1.267, the critical value of $t_{1\%}$ with 25 degrees of freedom being equal to 2.787. The standard deviation s_{xy} is 0.345. There is no significant difference between these data and those of Willis (*2*). These statistical data are presented in Table 1; the statistical parameters on Willis' data have been calculated by us.

The differences between the individual determinations in the whole set of 56 parallel determinations have been evaluated in another way. It is assumed that the oxalate-permanganate method gives the correct

Table 1. SERUM CALCIUM VALUES: CHEMICAL AND ATOMIC ABSORPTION (AAS) METHODS

Methods								
x	*y*	*No.*	*Intercept*	*Slope*	*s*	t_s	t_b	$t_{5\%}$
PRESENT INVESTIGATION								
Chem.	AAS-EDTA	28	0.55	0.953	0.345	1.565	1.267	2.056
WILLIS (*3*)								
Chem.	AAS*	5	0.14	1.006	0.663	0.385	0.016	3.185
AAS-EDTA	AAS*	22	0.25	1.013	1.788	0.124	—	2.086

*Serum deproteinized.

value; then the value obtained by atomic absorption is calculated as a percentage of this value. With this approach, the values obtained by atomic absorption average 101.3%, or 1.3% higher than the values obtained by the oxalate procedure (Table 2). The standard deviation of the differences is equal to 4.1%. The value for the significance of the difference between the two methods is 2.210. With 55 degrees of freedom the critical value of $t_{5\%}$ is 2.0002 and of $t_{1\%}$ 2.664. There is thus some possibility that by atomic absorption using 1% EDTA diluent the values for serum calcium are somewhat higher than those obtained by the Clark-Collip method. This difference is of no practical significance, however.

In Table 3 a more extensive set of data is shown, comparing serum

Table 2. CALCIUM DETERMINATION BY ATOMIC ABSORPTION, COMPARED WITH CHEMICAL METHODS

	No.	*Recovery (%)*	*Accuracy*	*V*
SERUM CALCIUM				
This study	5	100.1	—	1.60
Willis (*3*)	9	100.0	—	1.93
This study	56	—	101.3†	4.43
Willis (*3*)	5	—	99.6†	5.42
URINARY CALCIUM				
This study	4	96.5	—	3.96
Willis (*4*)	4	100.8	—	1.55
This study	17	—	99.6*	5.15
Willis (*4*)	15	—	100.7†	7.45

*Percentage in comparison with the oxalate gravimetric method.
†Percentage in comparison with the oxalate-permanganate method.

Table 3. Serum Calcium by Atomic Absorption as Compared with Oxalate-Permanganate Procedure

No. tests	*Serum (ml.)*	*Diluent*	$\bar{x}$	*s*
38	0.5	4.5 ml., 1% EDTA	100.1	±3.3
37	0.2	4.8 ml., 1% EDTA	101.0	±3.8

calcium determinations by atomic absorption with those by the oxalate-permanganate method.

The precision with which calcium can be determined, both by atomic absorption using 1% EDTA as the diluent and by the classical chemical method, is shown in Table 4. V is quite small for replicate determinations performed the same day, and is of the same order for both the instrument procedure and the classical chemical procedure. As might be expected, precision calculated from data collected over several days with a pooled serum as a sample is not as good. The V's for both methods are approximately 2.5 times as great as those obtained with replicate determinations performed the same day. A V of this order with the analyses performed on different days represents a quite acceptable degree of precision for this type of analysis.

In this regard, it is of interest to compare these coefficients of variation with those obtained for sodium and potassium by flame photometry with the Baird Associates flame photometer (*18*). These data are shown in Table 5. V for sodium and potassium, as well as for serum calcium by the oxalate-permanganate method, was calculated from data obtained on pooled sera used in our routine quality control program.

Since we adopted atomic absorption spectrophotometry as the routine procedure for serum calcium, it has been checked on two different occasions by the classical procedure of oxalate precipitation and per-

Table 4. Calcium Precision by AAS and Oxalate-Permanganate Methods

Method	*Solution*	*Concentration (mg./100 ml.)*	*Replication*		*V*
			Day	*No.*	
AAS	Standard	2.5	Same	4	1.25
AAS	Standard	5.0	Same	5	0.35
AAS	Standard	7.5	Same	5	1.11
AAS	Standard	10.0	Same	5	0.49
AAS	Standard	12.5	Same	5	0.42
AAS	Standard	15.0	Same	5	0.80
AAS	Serum	9.3	Same	6	0.55
OX-P	Serum	9.6	Same	6	0.63
AAS	Serum	9.4	Different	10	2.30
OX-P	Serum	9.2	Different	10	2.66

Table 5. Precision for Sodium and Potassium in Serum by Flame Photometry

Analysis	Concentration (mEq/L.)	No. of Replicates*	V
Sodium	141	5	2.58
Sodium	139	9	1.42
Sodium	137	8	2.63
Potassium	4.6	6	3.17
Potassium	4.8	8	1.37
Potassium	4.4	8	2.27

*Analyzed on different days.

manganate titration. In both instances essentially the same values for accuracy were obtained as those above: the chemical procedure yielded results equal to 98.4 and 97.3% of those obtained by atomic absorption spectroscopy.

In Urine

For the determination of calcium in urine, Willis found lanthanum chloride—a 1% (w/v) solution—to be the best diluent of several tried. This diluent seemed to eliminate the interference from phosphate better than did EDTA or strontium chloride. In preliminary studies with 1% EDTA as the diluent (Table 6), recovery of calcium added to urine appeared better than when 1% lanthanum chloride was used. However, upon more comprehensive testing, dilution with 1% lanthanum chloride (see Table 7) resulted in better agreement with the chemical procedure

Table 6. Recovery of Calcium Added to Urine

Diluent	Tests	Recovery (%)	s^2	s	V
EDTA	4	97.4	3.62	1.90	1.95
$LaCl_3$	4	96.5	14.62	3.82	3.96
$SrCl_2$	5	90.4	31.3	5.58	6.18

Table 7. Atomic Absorption vs. Chemical Methods for Urinary Calcium

Methods compared*	Diluent	Tests	Ratio (%)	s^2	s	V
AAS/Ox-G	$LaCl_3$	7	97.9	14.23	3.78	3.86
AAS/Ox-G	EDTA	7	82.6	—	—	—
AAS/Ox-P	$LaCl_3$	6	96.8	31.72	5.62	5.80
AAS/Ox-P	$LaCl_3$	4	100.8	33.42	5.78	5.73
AAS/Ox-P	$LaCl_3$	7	101.4	18.61	4.31	4.25
AAS/Ox-P	EDTA	7	83.0	30.7	17.5	21.08

*Ox-G indicates the chemical oxalate, precipitation, incineration, gravimetric method; Ox-P, the Taussky method (*20*).

than did dilution with EDTA. Thus our final method utilized a dilution with 1% lanthanum chloride.

Two different methods were used for the chemical determination of urinary calcium. One is the classical macrogravimetric procedure in which calcium is precipitated as the oxalate, isolated, incinerated, and finally weighed as the oxide. This procedure was performed for us at another laboratory (*19*). The other method is that described by Taussky (*20*), which is essentially an oxalate precipitation-permanganate titration procedure applied after the urine has been extracted with chloroform. In our own evaluation trials of Taussky's method, we were able in 7 trials to recover 96.3% of the calcium added to urine. The coefficient of variation was 3.06.

Recovery data and accuracy tests for atomic absorption versus chemical analyses are reported in Table 2, with comparable data from Willis (*4*).

In Diet and Stool

The diets analyzed were duplicates of those fed to volunteer pregnant patients who were subjects for a metabolic investigation. The diets were planned to be adequate for optimum nutrition during pregnancy. The stools were from the same patients.

For analysis, diets are first homogenized; samples of the homogenate are placed in weighed platinum dishes and then weighed. They are partially dried at 90°, then dried completely at 130°. The dried material is ashed at 550° in a Thermolyne 2000 furnace. When cool, the ash is dissolved in a few drops of nitric acid and transferred to a 25-ml. volumetric flask containing distilled water; it is then brought to volume with distilled water and appropriate dilutions made.

Stools are collected in a No. 5 Lily tub lined with an 8- by 4- by 18-in. polyethylene bag, which weighs about 7 gm. The stool is frozen in this bag, and the frozen stool and bag are weighed together; thus the weight of the stool is obtained. The frozen stool separates easily and cleanly from the polyethylene bag if it is necessary to remove it. In our metabolic studies, stools from a 48-hr. period are usually combined. To the combined stool as passed, 800 ml. of water is added. The single plastic bag containing the frozen combined stools is then encased in two more plastic bags of the same size. The bags are closed by twisting the open ends together, then folding the twisted ends over. Closure is maintained with a heavy rubber band wrapped around the folded-over ends. This package is placed in a 1-gal. paint can and the cover is closed. The can is then shaken for 15 min. on a paint mixer. A small sample of the re-

Table 8. Recovery of Calcium Added to Diet and Stool Homogenates (mEq)

Sample wt. (gm.)	Ca in Sample*	Ca added	Total Ca		Recovery (%)†
			Expected	Found	
DIET HOMOGENATE					
5.2658	0.184	0.055	0.239	0.241	100.8
4.1320	0.144	0.055	0.199	0.200	100.5
5.3061	0.185	0.055	0.240	0.245	102.1
4.6150	0.161	0.055	0.216	0.209	96.8
STOOL HOMOGENATE					
5.3295	0.421	0.055	0.476	0.463	97.2
5.7858	0.457	0.055	0.512	0.515	100.6
4.6709	0.369	0.055	0.424	0.425	100.2
4.9942	0.395	0.055	0.450	0.438	97.3

*By analysis, 1 gm. of diet was found to contain 0.034 mEq. Ca, and 1 gm. of stool homogenate, 0.079 mEq. of Ca.

†Average: for diet, 100.1%; for stool, 98.8%.

sulting homogenate is then weighed in a preweighed dish. The remainder of the sample preparation is the same as that for diet.

Studies of the recovery of calcium added to diet and stool are shown in Table 8. These data indicate the accuracy of the over-all process. For the final dilution, 0.5 ml. of the diluted solution to be analyzed is added to 4.5 ml. of 1% lanthanum chloride.

The diets and stools analyzed in this study are not necessarily representative of all possible diets or stools. Analysis of stools from patients receiving antacids like Amphojel,* which contains aluminum salts, may be subject to error because of the presence of these salts in the stool.

Magnesium

To measure the accuracy with which magnesium can be determined in serum and urine by atomic absorption spectrophotometry, only recovery experiments, constituting the so-called internal-standard method of evaluation, were employed. In the procedure usually used for serum magnesium, 0.2 ml. of serum was diluted with 5 ml. of diluent. As shown in Table 9, we have found, in agreement with the observations of Willis (*3, 4*), an enhancement of absorption when water is used as a diluent and acceptable recoveries of added magnesium when 0.25% strontium chloride is used as a diluent. Thus the strontium chloride seems to suppress the enhancement. A similar enhancement of absorption was observed when 15% TCA was used to precipitate the protein. This effect

*Wyeth Laboratories, Philadelphia, Pa.

Table 9. Recovery of Magnesium Added to Serum and Urine

Diluent	*Tests*	*Recovery (%)*	s^2	s	*V*
Serum					
$SrCl_2$	4	101.2	2.44	1.56	1.55
H_2O	4	107.8	2.74	1.65	1.53
TCA	4	107.9	13.63	3.68	3.41
Urine					
H_2O	5	99.4	8.31	2.88	2.90
$SrCl_2$	4	101.1	9.17	3.03	3.00

Table 10. Recovery of Magnesium from Diet and Stool Homogenates (mEq)

Sample wt. (gm.)	*Mg in sample**	*Mg added*	*Total Mg*		*Recovery (%)†*
			Expected	*Found*	
DIET HOMOGENATE					
4.8731	0.058	0.028	0.086	0.083	97
4.7229	0.057	0.028	0.085	0.083	98
4.9651	0.060	0.028	0.088	0.087	99
3.6761	0.044	0.028	0.072	0.073	101
STOOL HOMOGENATE					
5.4884	0.178	0.027	0.205	0.213	103.9
5.6433	0.183	0.027	0.210	0.210	100.0
4.9010	0.159	0.027	0.186	0.187	100.5
4.4241	0.143	0.054	0.197	0.209	106.1
6.5505	0.212	0.054	0.266	0.261	98.1

*By analysis, 1 gm. of diet was found to contain 0.012 mEq. of Mg, and 1 gm. of stool, 0.0324 mEq. Mg.

†Average: for diet, 99%; for stool, 101.6%.

Table 11. Coefficients of Variation for Magnesium by Atomic Absorption Method

Concentration	*No. of replicates*	*V*
STANDARDS, REPLICATED ON SAME DAY (MG./100 ML.)		
0.5	5	1.06
1.0	5	0.56
2.0	5	1.05
3.0	5	0.34
4.0	6	1.64
SERA, REPLICATED ON DIFFERENT DAYS (MEQ/L.)		
1.82	5	1.10
1.82	5	2.07
1.82	16	2.52

Table 12. Recovery of Magnesium Added to Urine with 0.25% Strontium Chloride or Water as Diluent (mg./100 ml.)

Urine dilution	*Mg in urine*	*Mg added*	*Total Mg*	*Mg found*	*Recovery (%)**
			STRONTIUM CHLORIDE		
1 to 100	0.64	0.5	1.14	1.17	103
2 to 100	1.34	0.5	1.84	1.86	101
3 to 100	1.96	0.5	2.46	2.55	104
4 to 100	2.66	0.5	3.16	3.06	97
			WATER		
0.4 to 100	0.31	0.2	0.51	0.49	96
0.5 to 100	0.38	0.25	0.63	0.63	100
1 to 100	0.73	0.50	1.23	1.23	100
2 to 100	1.43	0.50	1.93	1.88	98
3 to 100	2.0	0.20	2.20	2.28	104

*Average: with strontium chloride, 101%; with water, 100%.

was observed whether strontium chloride was present or not. The diluent used routinely for serum magnesium determination is 0.25% strontium chloride.

Recovery of magnesium from diet and stool homogenates is shown in Table 10. The final solutions used in these analyses consisted of 0.1 ml. of appropriately diluted ash solution in 5.0 ml. of water.

The precision with which magnesium can be determined is shown in Table 11. Again, as expected, the precision obtainable is much better when the replicate analyses are performed on the same day than when they are performed on different days.

Table 12 shows the recovery of magnesium from urine with 0.25% strontium chloride and with water used as diluents. Water as the diluent appears to give slightly better results statistically, but the difference is not significant. For the sake of simplicity, water is used as the preferred diluent.

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Automated, Simultaneous Microdetermination of Calcium and Magnesium by Atomic Absorption

Nathan Gochman and Harry Givelber

A procedure is described for the simultaneous, automatic determination of serum calcium and magnesium with the AutoAnalyzer, and a dual-channel, double-beam atomic absorption spectrophotometer, the IL Model 153. Advantages over existing procedures include a high rate of analysis (90 samples or 180 tests per hour) and low sample consumption (a total of 30 μl for both determinations). We have compared the new procedure with a manual one in which the Perkin-Elmer Model 303 atomic absorption spectrophotometer is used.

Availability of an atomic absorption spectrophotometer (AAS) with two channels (one monochromator and one filter position), the Instrumentation Laboratory Model 153, provided the starting point for investigation of a simultaneous, automated procedure for serum calcium and magnesium.

The work of Klein *et al.* (*1–3*) indicated the feasibility of the AutoAnalyzer with an atomic absorption detection system for determining serum cations. These authors investigated the use of a Techtron Model AA-3 single-beam, single-channel AAS combined with a double-dialysis manifold, which provided a protein-free diluted sample. Both serum calcium and magnesium could be determined with their system using two sample runs at a net rate of analysis of 20 to 30 samples per hour. Sample consumption would be approximately 1.2 ml, which limits the procedure's usefulness for pediatric specimens, on which electrolyte determinations are frequently required.

Zettner and Seligson (*4*), Pruden *et al.* (*5*), Trudeau and Frier (*6*), and Johnson and Riechmann (*7*), have demonstrated that accurate determinations can be made directly by atomic absorption in the presence of serum proteins. Rodgerson and Moran (*8*) reported a manual atomic absorption micromethod for calcium that requires 20 μl of serum and is based upon sample dilution and measurement without deproteinization. Their procedure was more reliable than a microfluorometric method. If deproteinization were unnecessary, an automated procedure of considerably higher sensitivity (lower sample consumption) would result, if recordings could also be made noise-free and the effects of serious burner clogging by protein could be eliminated.

This report describes the details of a combined AutoAnalyzer and IL 153 system that satisfies the requirements indicated above and correlates well with a manual atomic absorption procedure. Sample consumption at 90 samples per hour (sample to wash ratio, 1:1) is 30 μl, which fits the scope of automated microchemical determinations suitable for pediatric specimens as described by Mabry *et al.* (*9*).

Materials and Methods

Reagents and Standards

Lanthanum–butanol diluent (0.5 g of lanthanum per 100 ml of n-butanol, 40 ml/liter). Lanthanum oxide (La_2O_3), 5.85 g, (Alfa Inorganics, Inc., Beverly, Mass.), is dissolved in 25 ml of concd hydrochloric acid, then diluted with about 200 ml of de-ionized water; 40 ml of *n*-butanol is added and the solution is diluted to one liter with de-ionized water.

Stock calcium standard (100 mEq/liter). Dried calcium carbonate ($CaCO_3$), 5.0045 g, is dissolved in 4 ml of concd hydrochloric acid and diluted to one liter with de-ionized water.

Stock magnesium standard (50 mEq/liter). Mag-

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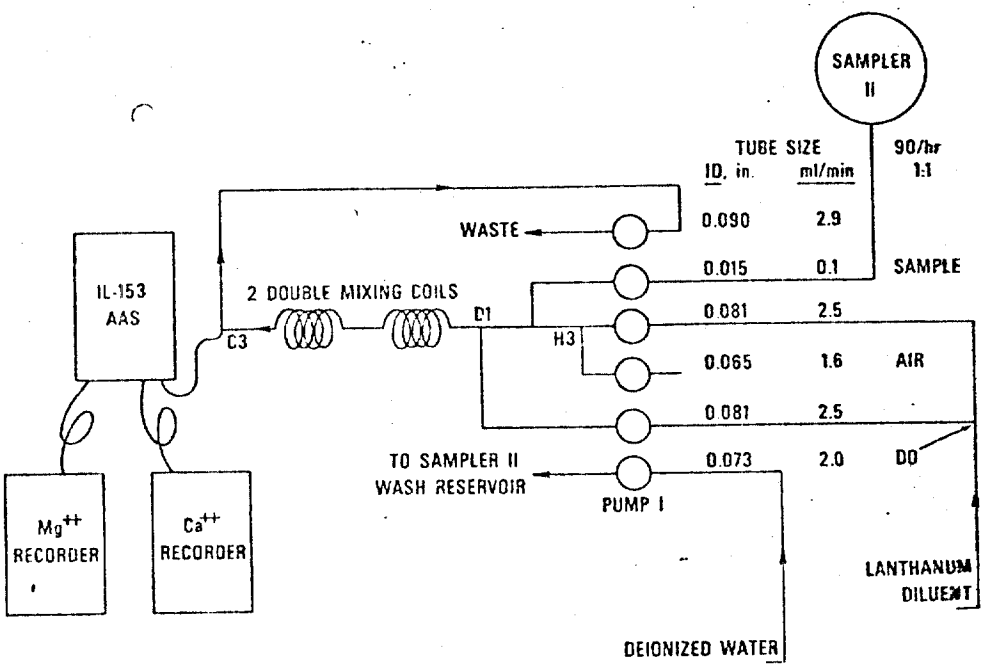

Fig. 1 Flow diagram of the arrangement for simultaneous automated determination of serum calcium and magnesium

nesium metal, 0.6080 g, is dissolved in 2 ml of concd hydrochloric acid and diluted to one liter with de-ionized water.

Stock saline (17 g/100 ml). Seventeen grams of sodium chloride is diluted to 100 ml with de-ionized water.

Combined working standards

Ca, mEq/l	Mg, mEq/l	Ca stock, ml	Mg stock, ml	NaCl stock, ml
2.0	0.5	2.0	1.0	5.0
4.0	1.0	4.0	2.0	5.0
5.0	1.5	5.0	3.0	5.0
6.0	2.0	6.0	4.0	5.0
7.0	2.5	7.0	5.0	5.0
8.0	3.0	8.0	6.0	5.0

Aliquots of the stock solutions, as indicated, are combined and diluted to 100 ml with de-ionized water.

Instrumentation

Atomic absorption spectrophotometer. The Instrumentation Laboratory (Watertown, Mass.) Model 153 AAS used for this investigation was equipped with a magnesium hollow-cathode lamp in the turret position (Monochromator optical path), and a calcium hollow-cathode lamp in the fixed position (422.7 nm interference filter optical path). The burner used was a Boling-type with a three-slot head and 10-cm light path. This AAS has a linear recorder output, and when operated in the 2.5-scale position reads directly in absorbance units.

Recorders. Two SR recorders (E. H. Sargent Co., Chicago, Ill.) with variable range selector switches (up to 125 mV) were used. Chart speed was set at medium, 1-in./min. The IL 153 servo potentiometers required grounding for zero adjustment of these recorders.

Automatic sample feed and dilution. Technicon AutoAnalyzer modules were assembled as shown in the flow diagram (Figure 1). The Sampler II is operated with the 90-per-h cam, and a sample-to-wash ratio of 1:1. A check of the actual aspiration time of each cam lobe should be made as discussed by Young *et al.* (*10*).

Operating Procedure

Preliminary adjustment of the IL-153 AAS requires the following fixed settings:

Test Mode—A, B; Channel A milliamp setting—4; Channel B—5; Scale—2.5; Curve correct—0; Calibration—full counterclockwise.

After "Power" and "Hollow Cathode" switches are turned on, the Channel A monochromator is peaked for maximum energy by rotation of the wavelength dial (nominal magnesium absorption peak at 285.2 nm), and energy-balance meters are adjusted. Monochromator slit setting is 160 μ.

The flame is ignited and the AutoAnalyzer manifold connected to the atomizer. Fuel (acetylene) pressure is adjusted to 4 lb/in.^{2} and oxidant (compressed air) pressure to 12 lb/in^2. The AAS zero controls are adjusted so that the absorbance reads zero on the digital displays. The recorder zero controls are then adjusted to 5 divisions on the 100-division linear chart paper.

With the instrument sampling the highest combined working standard, the recorders are each set to about 90 lines while the steady state is plotted.

Samples are placed in microcups of 0.5-ml

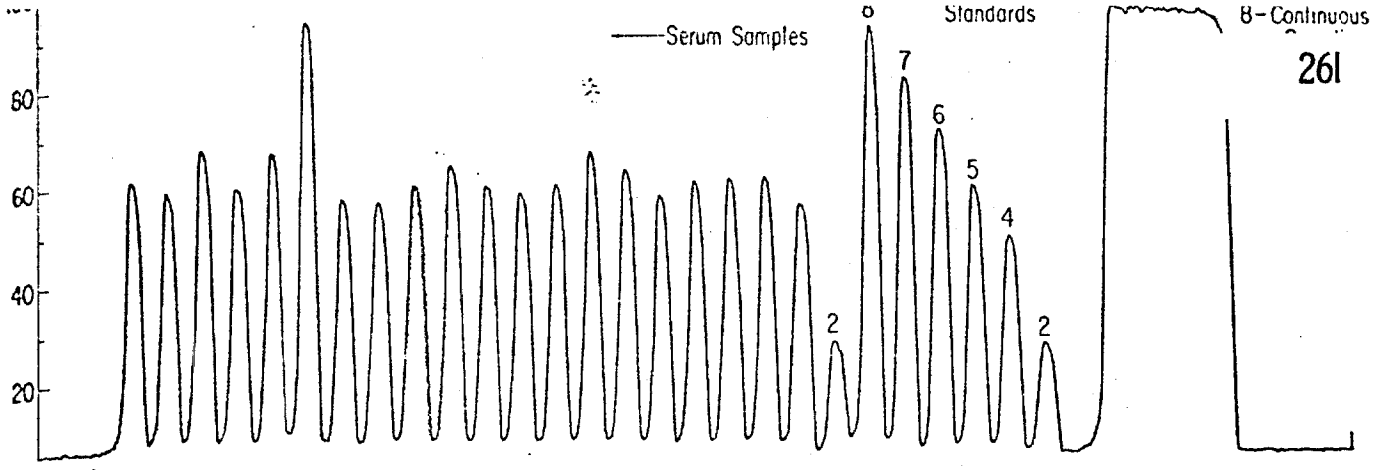

Fig. 2. Representative recording of calcium determination, showing steady state, aqueous standards, and serum specimens

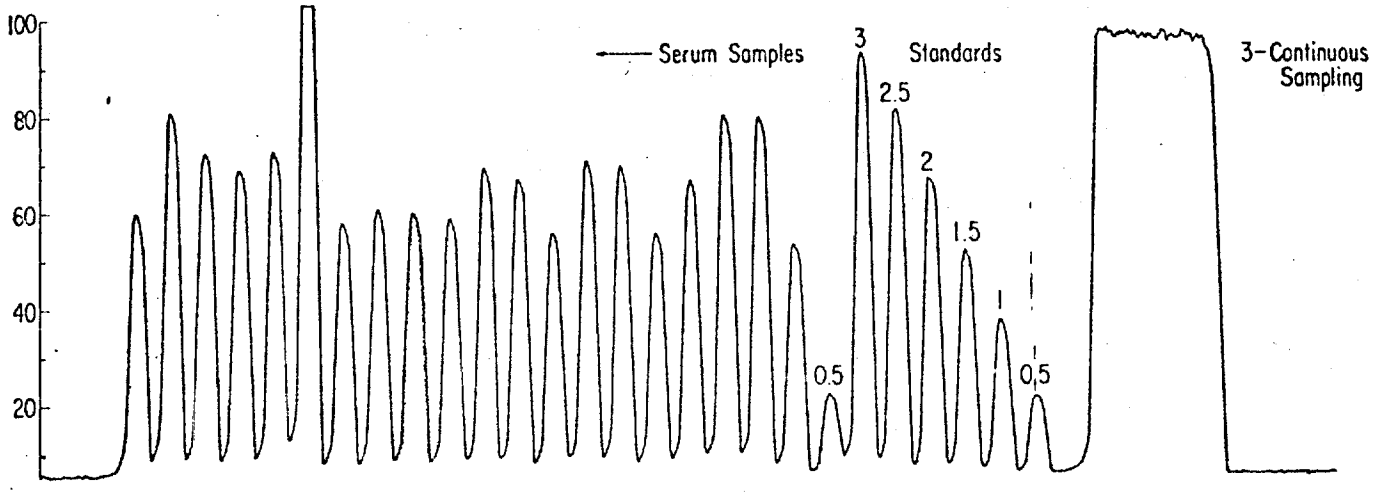

Fig. 3. Recording of magnesium determination, made simultaneously with that in Fig. 2

capacity. As little as 100 μl of serum (first marked line) may be used if the sample pickup probe is carefully positioned.

Standards and samples are analyzed, and standard curves are prepared on linear graph paper for calculation of results for serum.

Results

Figure 2 shows a recording of the calcium-monitoring channel, including steady state of 8 mEq/liter, aqueous standards (including lowest following highest to note interaction level), and a number of random serum specimens. The concurrent magnesium recording is shown in Figure 3.

Occasionally, a serum magnesium value is extremely supranormal if a patient has been treated with magnesium salts, as illustrated by the off-scale specimen shown in this recording. Such samples are diluted with an equal volume of saline and reanalyzed.

The calibration curve for calcium is linear to about 7 mEq/liter, beyond which it deviates slightly downward (Figure 4). Magnesium deviates from linearity by about 10% at 3 mEq/liter (Figure 5).

The within-run precision of the automated procedure was tested by analyzing a serum pool 30 times consecutively in a single run; the results are listed in Table 1.

Frozen aliquots of the serum pool were analyzed on 20 consecutive days during routine operation of the method. The results, which give a measure of the day-to-day reproducibility of the procedure, are shown in Table 2.

Table 1. Within-Run Precision of Automated Calcium and Magnesium Determination on a Serum Pool Sampled 30 Times

	Calcium	Magnesium
Mean, mEq/liter	4.59	1.55
SD	0.052	0.022
CV, %	1.1	1.4
Range	4.40–4.70	1.48–1.59

Table 2. Day-to-Day Precision of Calcium and Magnesium Determination on Aliquots of Frozen Serum Pool (20 Daily Analyses)

	Calcium	Magnesium
Mean, mEq/liter	4.53	1.57
SD	0.083	0.042
CV, %	1.8	2.7
Range	4.40–4.75	1.52–1.67

The automated procedure described here was compared with the routine laboratory procedure (4) that uses a Perkin-Elmer Model 303 AAS and a semiautomatic diluter for 50-fold dilution of serum. Diluting reagent for the Perkin-Elmer method is a mixture of 0.5 g of lanthanum, 6 ml of butanol, and 94 ml of water. Results of the correlation are shown in Table 3. The calcium results agree very well, while a small but significant difference in means for magnesium is evident. This difference is related to standardization of the two procedures and will be discussed later.

Table 4 summarizes analytical data obtained from the analysis of two types of commercial lyophilized control serum by the two procedures.

Discussion

The first consideration in setting up the dual-channel Instrumentation Laboratory Model 153 Atomic Absorption Spectrophotometer was the selection of either calcium or magnesium analysis with the grating monochromator. The numerous spectral lines in the 285.2 nm vicinity that could cause interference with the determination of magnesium demanded that magnesium be used with the higher resolution grating. A high-quality interference filter, of 422.7 nm nominal peak wavelength with a 6 nm band-pass, was used for the calcium analytical channel.

Factors influencing the determination of calcium on this instrument were reported by Bowers *et al.* [CLIN. CHEM. 14, 846 (1968), abstract]. These authors used strontium as an internal standard and measured the calcium–strontium ratio. Because of the many unsuspected abnormalities of magnesium metabolism observed in our patients, we thought it more desirable to use the second channel to provide a simultaneous magnesium determination. The results of the precision studies indicated that adequate stability could be obtained without resorting to an internal standard technique. The major instability factor that might be introduced is variation in hollow-cathode lamp intensity, but this is satisfactorily compensated by the double-beam operation (flame path *vs.* reference air path) of each analytical channel.

To satisfy the requirements for a high rate of analysis and low sample consumption, a nondialysis AutoAnalyzer manifold was investigated. The use of an aqueous lanthanum diluent with the manifold shown in Figure 1 resulted in rapid-forming deposits in the slot of the burner during aspiration of serum samples. This caused a ragged flame and a drop in sensitivity within 30 min.

The presence of butanol in the lanthanum diluent greatly reduced the rate of deposit accumulation, but also caused a slight decrease in wash-out characteristics of the manifold *vs.* the aqueous reagent. Butanol, 4 ml/100 ml, was chosen for

Table 3. Comparison of Results for Serum Calcium and Magnesium Obtained by Automated System with IL-153/AA and by Manual Atomic Absorption with PE-303

	Calcium, mEq/liter		Magnesium, mEq/liter	
	IL-153/AutoAnalyzer	PE-303	IL-153/AutoAnalyzer	PE-303
No. samples	160	160	124	124
Mean, mEq/liter	4.69	4.71	1.64	1.55
SD	0.51	0.49	0.26	0.25
Range	2.85–6.85	2.85–6.60	0.65–2.64	0.61–2.57

Table 4. Comparative Analysis for Ca and Mg in Commercial Lyophilized Control Sera by Automated Procedure (IL-153/AA) and Manual Procedure (PE-303)[a]

Sample	Calcium, mEq/liter			Magnesium, mEq/liter		
	Label	IL-153/AutoAnalyzer	PE-303	Label	IL-153/AutoAnalyzer	PE-303
Calibrate[b] 1	3.75	3.68	3.63	1.32	1.31	1.20
Calibrate 2	5.25	5.08	5.16	1.73	1.65	1.57
Calibrate 3	6.75	6.68	6.68	2.14	2.06	1.98
Monitrol[c]	4.84	4.77	4.75	2.5	2.32	2.26

[a] Values are av. of duplicate or triplicate determinations.
[b] "Calibrate" is a product of Warner-Chilcott Laboratories, Morris Plains, N. J. Lot No. 677108.
[c] "Monitrol" is a product of Dade Reagents, Miami, Florida. Lot No. 95-D.

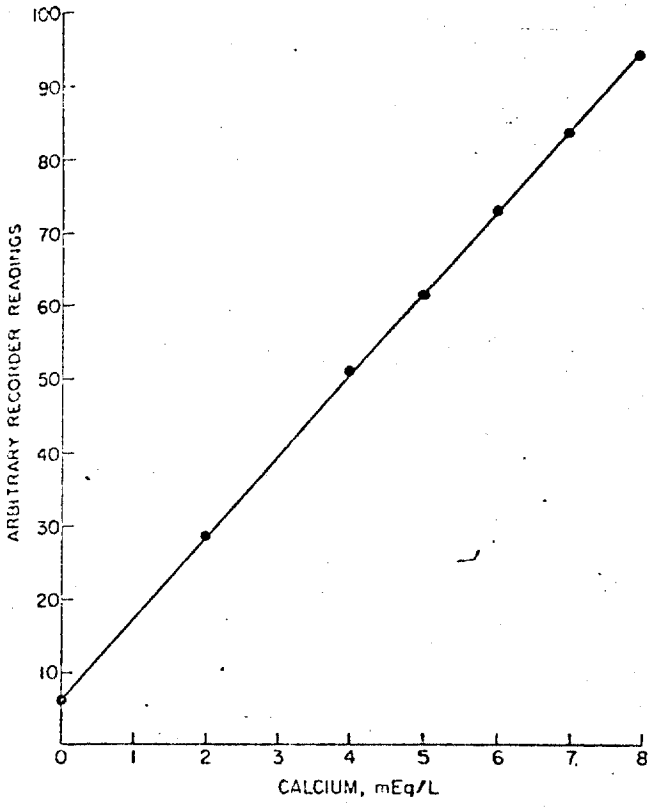

Fig. 4. Calibration curve for serum calcium, plotted on linear graph paper

the procedure since this concentration permitted 96–97% steady state to be attained for both aqueous standards and serum at 90 samples per hour and a sample-to-wash ratio of 1.

Over 200 serum specimens have been analyzed in a single run with this reagent without significant deterioration of the flame characteristics. The burner head is cleaned every day by immersing it in 10% nitric acid for 15 min or overnight.

Butanol also enhances magnesium absorbance and, at the 4% concentration used, produced a 30% increase in sensitivity compared to the reagent without butanol. There was no measurable effect on calcium absorbance; therefore, the effect of butanol on magnesium is apparently a direct enhancement rather than merely an increase in atomization efficiency, which would have affected both.

The AutoAnalyzer manifold was set up to provide a differential input of diluted sample to the AAS atomizer. The AAS is equipped with a variable aspiration-rate control capable of drawing between 0 and 10 ml/min of a solution open to the atmosphere. This was adjusted to draw from 5 to 6 ml/min, for optimum recordings when the AAS was connected to the AutoAnalyzer manifold delivering approximately 4 ml/min.

Absorbance values were about 0.700 for 3 mEq of magnesium per liter and about 0.150 for 8 mEq of calcium per liter with the manifold as described. These values were obtained with an actual manifold dilution factor of 42 compared to a nominal dilution of 50. This was c
comparing readings obtained by d
tion into the AAS of (*a*) an accurate
tion of the standard and (*b*) the diluted effluent collected from the AutoAnalyzer during sampling of the combined standard.

When the correlation study reported in Table 3 was completed, the aqueous standards used for the respective procedures were compared with particular reference to the magnesium contents. Lithium was included in the aqueous standards for the Perkin-Elmer procedure because of occasional determinations of lithium in serum. However, lithium produced no interfering effects on the determination of either calcium or magnesium. New standards using magnesium metal, and high-purity magnesium sulfate were prepared. These agreed well with the automated system standards, but were 5% lower in concentration than the standards used for the data obtained on the Perkin-Elmer apparatus. This accounts for the lower magnesium values obtained on the Perkin-Elmer apparatus for the commercial control sera and individual serum samples when compared with the IL/AutoAnalyzer system.

After several weeks of routine operation of the automated procedure, we observed that occasional

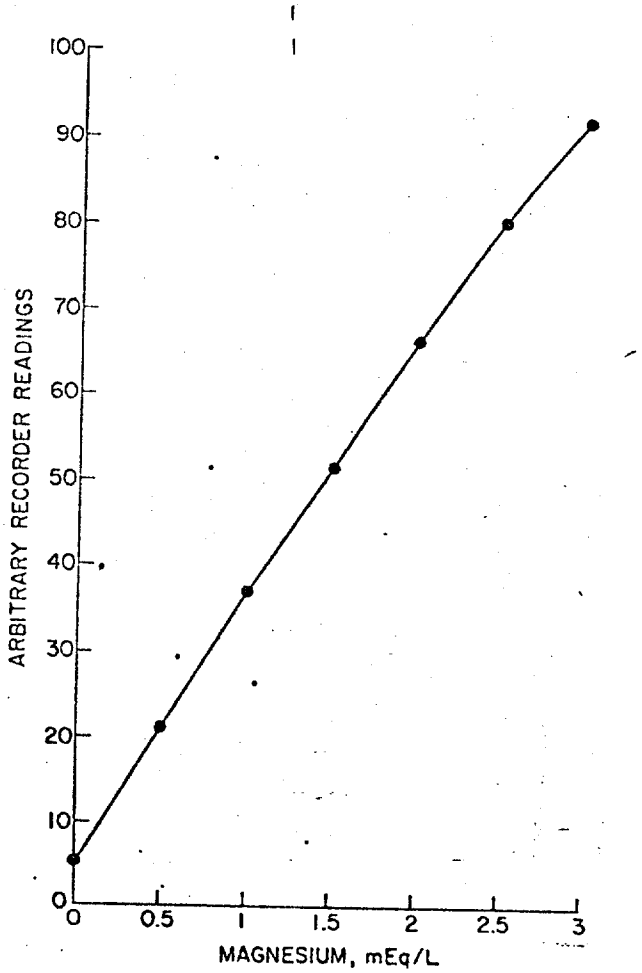

Fig. 5. Calibration curve for serum magnesium, plotted on linear graph paper

Table 5. Comparison of Sodium Background Effects with Two Calci Interference Filters (Calcium, mEq/liter)

Sample	Label	Old calcium filter: Calcd. vs. regular standards, Found	Old calcium filter: Calcd. vs. regular standards, % label	Old calcium filter: Calcd. vs. standards without NaCl, Found	Old calcium filter: Calcd. vs. standards without NaCl, % label	New filter[a], Found	New filter[a], % label
HSP[b]	6.35	5.65	89	6.40	101	6.50	102
LSP[b]	3.00	2.30	77	2.95	98	3.00	100
Calibrate 1	3.75	3.40	91	4.00	107	3.80	101
Calibrate 2	5.25	4.90	93	5.60	107	5.10	97
Calibrate 3	6.75	6.50	96	7.30	108	6.85	101
Monitrol	4.84	4.45	92	5.10	105	4.80	99

[a] Standard curves with and without NaCl indistinguishable, therefore only one set of calculated data.
[b] HSP and LSP are aqueous calcium solutions prepared without NaCl and used as controls in the Clinical Chemistry Laboratory. Other samples identified in legend to Fig. 4.

lyophilized serum controls were producing results for calcium 5 to 10% lower than usual. When this problem became more consistent, we investigated and found the effect to be related to a change in transmission characteristics of the interference filter.

Samples were analyzed with the calcium filter currently used and a newly received filter, using standards prepared as described and another series of standards in which the 0.85% sodium chloride was omitted. The old calcium filter gave absorbance readings for the sodium-containing standards that were considerably higher than those prepared without sodium. As can be seen from Table 5, results for control serum were low when calculated against the sodium-free standards. Apparently, the sodium background effect is modified in serum, since the sodium level is approximately the same as in the standards but compensation is not achieved.

With the new filter, the two sets of standards were indistinguishable, as was the case with the old filter initially, and when we used the Channel A monochromator. Calculated data for the control sera agreed well with label values. We suspect that delamination of the initial filter caused the abnormal sensitivity to sodium, but we are not sure why this occurred.[1]

[1] Subsequent examination of the old filter revealed missing epoxy sealer between the glass and holder, which could have admitted stray light. The new filter has been used for nearly a year without any difficulty.

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3. Klein, B., Kaufman, J. H., and Oaklander, M., Automated atomic absorption spectrophotometry. III. Calcium in urine and spinal fluid. CLIN. CHEM. 13, 797 (1967).

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ANTIDOTES FOR STRYCHNINE POISONING*

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Recently Aikman[1] has called attention to the importance of strychnine as a cause of fatal poisoning in children. He adds three cases to the long list of fatalities already attributed to this alkaloid. Statistical studies[2] on the subject indicate further that strychnine is the commonest cause of poisoning among children. In most instances the strychnine is obtained from sugar-coated cathartic or tonic pills purchased by adults for their own use but carelessly left within the reach of children. This source of poisoning can eventually be eliminated by legislation against the dispensing of strychnine in this form. Time will be required, however, to bring about this change, and in the meantime accidental fatalities will continue. There will always be the occasional cases of accidental or intentional poisoning among adults.

The methods of treatment for strychnine poisoning now in use are apparently not very effective. They consist of emetics or the use of the stomach tube for the recovery of unabsorbed strychnine; but because the latter procedure precipitates convulsions, it is frequently necessary to anesthetize the patient while it is being carried out. Treatment is then directed toward the control of convulsions to avoid fatal asphyxiation or exhaustion, and to this end antispasmodics and volatile anesthetics are commonly employed. The anesthetics, however, are prone to cause respiratory failure when given in amounts sufficient to control convulsions; their use is thus attended with some risk. Morphine is said to augment rather than to allay convulsions.

In experimental investigations of strychnine poisoning, Githens and Meltzer were able to save dogs that

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1. Aikman, John: Strychnine Poisoning in Children, J. A. M. A. 95:1661 (Nov. 29) 1930.

2. Statistical Bulletin, Metropolitan Life Insurance Company 6:1-2 (April) 1925.

had received twice the surely fatal dose by the employment of anesthesia, intratracheal insufflation and the injection of salt solution. Fardera and Gentilucci, and also Osterwald found that rabbits and guinea-pigs would survive one and a half times the fatal dose if they were allowed to inhale oxygen. Neither of these methods has come into any general use.

The experimental work reported here was carried out mainly on rats, with a few additional experiments on dogs. The method of procedure was such as to allow simple graphic representation of the observations. The successive rats in a series were given increasing doses of strychnine varying from a nonfatal amount up to five times the amount ordinarily fatal. The antispasmodic drug under study was also administered but in a uniform amount throughout the series. Thus the only variable was the strychnine. The dose of antispasmodic drug, however, was different for each separate series of rats. Thus the series themselves provided the second variable. The procedure was in principle one of titration in which the death or recovery of the rat served as an indicator of the effectiveness of the antidote.

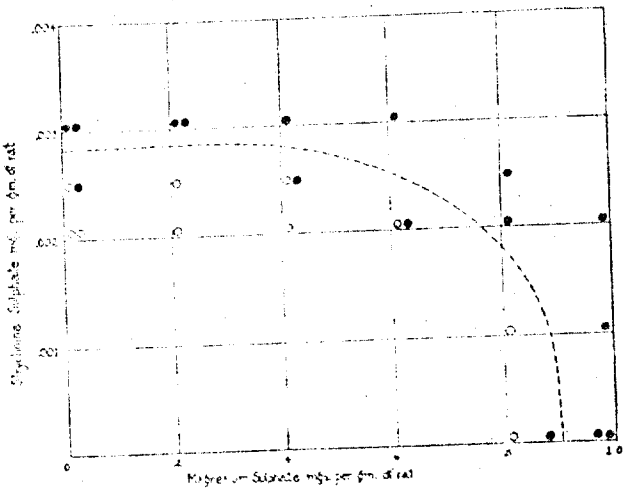

Chart 1.—Magnesium sulphate administered to rats poisoned with strychnine. The rats represented by black dots died; those represented by circles lived. The dose of strychnine and apomorphine given any one of the rats is indicated by the points on the abscissa and ordinate corresponding to the rat. Magnesium sulphate is not an antidote for strychnine.

EXPERIMENTS WITH MAGNESIUM SULPHATE AS AN ANTISPASMODIC

The results obtained with the use of magnesium sulphate are given in chart 1. The observations are essentially the same as those of Underhill and Wood[3] but, since in their experiments the strychnine was administered intramuscularly, their doses are somewhat larger than those reported here, which were given intraperitoneally. In this series of experiments the magnesium sulphate was administered before the strychnine was given. It was necessary to do so in the rats because the extremely rapid action of large doses of strychnine on these animals allowed no time to make subsequent administrations; administration of two or three times the surely fatal dose was followed by convulsions and death in from three to four minutes, and thus often before the antidote could be given. The action is slower in large animals; two or three times the lethal dose produces death in from fifteen to thirty minutes in dogs and after a much longer time in human beings. The difference in time depends on the more intense metabolism and more rapid circulation in small as compared with large animals.

In the experiments recorded in chart 1, twenty-three rats were injected intraperitoneally with amounts of strychnine varying from 0.001 to 0.004 mg. per gram of body weight. About half the rats given 0.0025 mg. per gram died; the others recovered. All those given less than 0.0025 mg. per gram recovered, and all those given 0.003 mg. or more per gram died. Therefore, 0.003 mg. per gram of rat was taken as the absolutely lethal dose. Magnesium sulphate solution injected intraperitoneally causes unconsciousness in rats in doses of 0.8 mg. per gram of rat and death in doses of 0.9 mg. or over. As seen in chart 1, the use of magnesium sulphate did not diminish the toxicity of strychnine nor did strychnine diminish the toxicity of magnesium sulphate.

USE OF APOMORPHINE AS AN ANTISPASMODIC

The use of apomorphine as an antispasmodic in strychnine poisoning was suggested by Dr. James S. Martin of Waterbury, Conn., who has used this drug successfully in three cases of strychnine poisoning. Dr. Martin has kindly allowed us to present the data from these cases:

CASE 1.—A child, aged 4 years, had swallowed an indefinite quantity of tablets of strychnine sulphate. Convulsions had started about one hour before medical treatment commenced. At the time of observation, convulsions were of extreme severity, with opisthotonos. Attempts to inject chloral hydrate by rectum started a convulsion, and the liquid was expelled violently. Death seemed imminent from respiratory failure.

One-tenth grain (6.5 mg.) of apomorphine was injected subcutaneously, and this procedure precipitated a violent convulsion, followed a minute later by one of less severity. At the end of three minutes, sweating commenced; the patient relaxed. During the next ten minutes there were a few spasmodic movements, but these passed off and there were no further convulsions. The patient did not vomit.

CASE 2.—A woman, aged 23, took strychnine with suicidal intent, as was later stated "a whole boxful" of one-thirtieth grain (2 mg.) tablets. Convulsions were well marked when medical treatment was commenced. A tap on the side of the bed was followed by a violent convulsion, accompanied by loud expiratory grunts, bursts of flatus and discharge of urine. There was extreme opisthotonos.

One-fifth grain (13 mg.) of apomorphine was injected subcutaneously, and precipitated a convulsion. As in the first case, sweating and relaxation followed; some spasmodic movements continued for one hour. One-tenth grain of apomorphine was then given. There were no further convulsive movements. The patient did not vomit. She recovered fully.

CASE 3.—A boy, at boarding school, in a fit of despondency decided to commit suicide. He stole from the school infirmary a bottle containing 100 one-tenth grain strychnine tablets. They were of the red sugar-coated variety. He carried them to his room, where he swallowed the entire hundred, washing them down with a glass of water. After sitting alone for twenty minutes, during which no symptoms of poisoning occurred, he decided that he had been mistaken in his choice of poison. He walked downstairs and took his place with the rest of the students in the study hall. Almost immediately he fell to the floor in a violent convulsion. A stomach tube was passed and a small amount of pinkish fluid was removed. Violent convulsions prevented any further attempt to wash out the stomach.

Fifteen minutes later, one-tenth grain of apomorphine was administered subcutaneously. The effect was the same as in the two cases previously cited. Twitching persisted for an hour and was controlled by the administration of an additional

3. Underhill, F. P., and Wood, E. C.: J. Pharmacol. & Exper. Therap. 3:67 [illegible] 1920

tenth grain of apomorphine. The patient did not vomit. recovered fully.

In commenting on these observations, Dr. Martin :

The first case, I feel quite sure, would have terminated ...ly without the use of apomorphine, as is quite probable in ...last two cases also). In all three cases the action of the ...g was spectacular. The almost immediate cessation of ...valsions following the administration of the apomorphine ...no room for doubt that it acted in a specific manner against ...rychnine.

EXPERIMENTS WITH APOMORPHINE AS AN ANTISPASMODIC

The antispasmodic and sedative effects of apomorphine have been observed by others,[4] and the drug has been used occasionally with these ends in view, but owing to its more striking action as an emetic it is used almost exclusively for that purpose.

Rats are unable to vomit, but following injections of apomorphine they exhibit actions indicating disturbance in the motility and secretion of the alimentary tract. After doses of from 0.033 to 0.066 mg. of apomorphine, they salivate freely and move their jaws in a constant chewing motion. They bite everything within their reach; they eat the feces in their cage and chew at their own tails or even ear skin from their bodies. At the same time the animals breathe violently, are restlessly active, turning in circles; but gradually the legs become stiff and their movements awkward. Paralysis develops, and death follows from doses of 0.13 mg., resulting from respiratory failure.

In testing apomorphine as an antidote for strychnine, the same procedure was followed as in the use of magnesium sulphate.

Chart 2 presents the results of experiments on twenty-nine rats given strychnine and apomorphine, thirteen given strychnine alone, and three given apomorphine alone. The use of apomorphine brought about

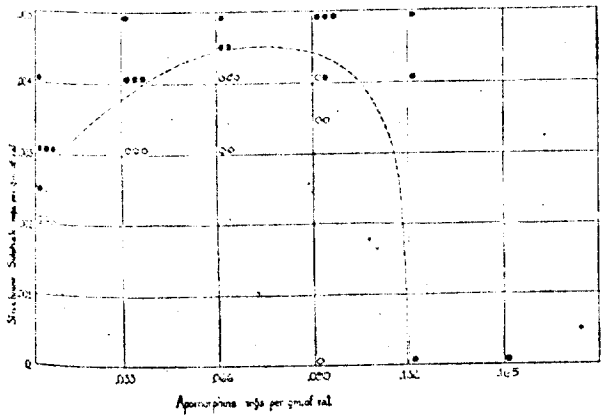

Chart 2.—Apomorphine administered to rats poisoned with strychnine: Presentation as in chart 1. Apomorphine is an antidote for strychnine, saving rats from death provided the dose of strychnine does not exceed twice the amount lethal for untreated rats. Strychnine does not act as an antidote for apomorphine.

recovery after the administration of slightly less than twice the fatal dose of strychnine. Strychnine, however, did not serve as an antidote to apomorphine.

Five experiments were performed on dogs to determine the effects of apomorphine in controlling strychnine convulsions in these animals. The lethal dose of strychnine for untreated dogs lies between 0.6 and 0.7 mg. per kilogram. The higher value was taken as the absolutely lethal dose. Intraperitoneal injection of this amount into one dog produced stiffness of the legs and arching of the back in five minutes, convulsions in ten minutes, and death in twenty-three minutes.

In a second dog treated in the same manner, stiffness and arching of the back developed in five minutes. At this time it was given 1 grain (0.065 Gm.) of apomorphine intraperitoneally. Dogs are less sensitive to apomorphine than are human beings, and doses as high as 3 grains (0.2 Gm.) can be tolerated. Strychnine convulsions did not develop in the dog. It vomited and for a half hour was restless, walking in circles. At the end of an hour the dog appeared normal but sleepy and thereafter did not show any ill effects from the drugs.

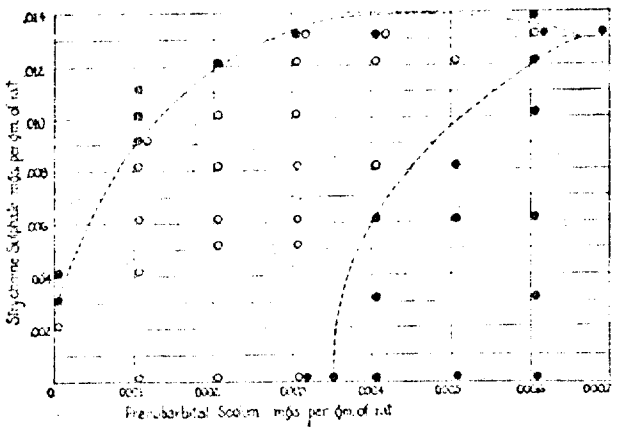

Chart 3.—Phenobarbital sodium administered to rats poisoned with strychnine: Presentation as in charts 1 and 2. Phenobarbital is an antidote for strychnine, saving rats after four to five times the amount lethal for untreated rats. Strychnine acts as an antidote for phenobarbital sodium, saving rats after twice the amount lethal for untreated rats.

A third dog was given double the absolutely lethal dose of strychnine (1.4 mg. per kilogram), and ten minutes later, when convulsions were definite and severe, 1 grain of apomorphine was injected intraperitoneally. The dog vomited and the convulsions ceased, but the dog remained hypersensitive to sensory stimulation for an hour, at which time this reaction disappeared and the dog appeared depressed but not otherwise abnormal.

The fourth and fifth dogs were each given three times the absolutely lethal dose of strychnine (2.1 mg. per kilogram). In both cases, extremely violent convulsions developed within two minutes. The convulsions were not controlled by the administration of 1 grain of apomorphine, and the animals died in nine and in twenty-three minutes, respectively, after the administration of strychnine.

EXPERIMENTS WITH PHENOBARBITAL SODIUM AS AN ANTISPASMODIC

Phenobarbital sodium has been used extensively as an antispasmodic in nervous disorders.[5] In testing phenobarbital sodium as an antispasmodic for strychnine convulsions, we followed the same procedure as that for apomorphine. The results, which are presented in chart 3, show that the lethal dose of phenobarbital for rats lies between 0.3 and 0.4 mg. per gram of rat. With less than a third of this lethal dose, recovery occurs after the administration of three times the absolutely

4. [illegible] Wchnschr. 29: 501 (Nov. 5) 1927.

5. Schoenborn, S.: Fortschr. d. Therap. 6: 178-180 (March 25) 1930. Banerjee, H.: Indian M. Rec. 50: 115-117 (Feb.) 1930. Kogan, J. M., and Mirelson, L. A.: Ztschr. f. d. ges. Neurol. u. Psychiat. 118: 621-633, 1929.

lethal dose of strychnine, and with the minimal lethal dose of phenobarbital, recovery occurs after the administration of from four to five times the absolutely lethal dose of strychnine. Furthermore, with large amounts of strychnine, recovery occurs after the administration of twice the lethal dose of phenobarbital.

There is thus apparently a true antagonism between these two drugs; such was not the case with apomorphine, for strychnine did not induce recovery when the ordinary lethal dose of apomorphine was given.

To study further the antidotal properties of phenobarbital sodium in strychnine poisoning, five experiments were performed on dogs.

The first dog was given twice the absolutely lethal dose of strychnine intraperitoneally (1.4 mg. per kilogram) and, at the same time, 0.1 Gm. of phenobarbital sodium per kilogram. Convulsions did not develop; the dog was slightly hypersensitive to sensory stimulation, but in twenty minutes this effect had passed off and the dog became sleepy. It slept quietly for ten hours and awoke apparently normal.

The second dog was given an excessive dose of phenobarbital, 0.12 Gm. per kilogram. Within twenty-five minutes the dog was unconscious and could not be roused by violent shaking. At this point, three times the lethal dose of strychnine was given (2.1 mg. per kilogram) without any appreciable effect on the dog. The dog was still asleep forty-eight hours later, and for the following twelve hours was awake but dull. Its behavior returned to normal after eight hours.

The third dog was given 0.08 Gm. of phenobarbital sodium per kilogram and thirty minutes later four times the lethal dose of strychnine (2.8 mg. per kilogram). Five minutes later it had a slight convulsion and several others of short duration in the hour following. None of these convulsions were of sufficient severity to endanger the animal's life. The dog finally fell asleep, and the next day was weak but not otherwise abnormal.

The fourth dog was given 0.3 Gm. of phenobarbital sodium per kilogram. There was some delay in administering the strychnine, and the dog died of respiratory failure thirty-two minutes after the injection of the phenobarbital.

The fifth dog was given the same large dose of phenobarbital sodium as in the case of dog 4; five minutes later it was unconscious and in ten minutes respiration was apparently failing. Twice the lethal dose of strychnine was administered with immediate benefit. The dog was alive ten hours later but unconscious. The lethal dose of strychnine was given and this was repeated three hours later. Following the last dose—altogether four times the lethal dose of strychnine within twenty-four hours—the dog awoke and was able to stand. The following day it appeared normal.

CONCLUSIONS

1. Magnesium sulphate does not prevent or even diminish strychnine convulsions in rats. It is not an antidote for strychnine.

2. Apomorphine controls convulsions in rats and dogs. It allows recovery after approximately twice the lethal dose of strychnine, but not when the dose is three times the lethal amount. Strychnine does not antagonize apomorphine or even diminish its toxicity for rats.

3. There are reported three cases in which the use of apomorphine was followed by recovery in human beings who had taken presumably lethal amounts of strychnine.

4. Phenobarbital sodium controls strychnine convulsions in rats and dogs. Recovery follows the administration of five times the lethal dose of strychnine.

5. A true antagonism between the actions of phenobarbital sodium and strychnine is indicated. Rats and dogs that have received amounts of phenobarbital as high as three times the lethal dose may be saved by the administration of amounts of strychnine which by themselves would be fatal.

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11187 P

Effect of Magnesium Sulfate on Serum and Peritoneal Fluid Calcium, Magnesium, Inorganic Phosphorus.*

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Four adult dogs were given intraperitoneal injections of 2.5% dextrose and 0.9% NaCl solution in the dosage of 100 cc per kg. After 16 hours, 20 cc of blood were withdrawn from the femoral artery and 20 cc of fluid from the peritoneum. Previous observations[1] have revealed that the serum and peritoneal fluid Ca, Mg and P are in equilibrium at this time. Fifty cc of a 25% solution of $MgSO_4$ were injected intramuscularly (3 cases) or subcutaneously (1 case). Blood and fluid samples (20 cc each) were obtained ½, 1, 1½, 2, 3, and 4½ hours after injection of the $MgSO_4$, and were examined for their Mg, Ca and inorganic P content.

Magnesium. The control values for serum Mg were 1.82-2.46 mg %. The maximum values after injection of magnesium ranged from 8.9 (subcutaneous injection) to 16.1 mg %, the peak being reached in 1-2 hours, with a subsequent decline.

The control values for peritoneal fluid Mg were 1.5-1.67 mg %. These increased in each instance, to values of 6.4-12.2 mg %, the peak being reached ½-1½ hours after the peak of serum Mg concentration.

Calcium. The control serum calcium values were 9.45-12.1 mg %. These fell in each instance, to minimum levels of 6.39, 7.0, 7.6 and 8.92 mg %, the last in the animal injected subcutaneously. The maximum drop in serum Ca concentration (2.31-4.5 mg %) occurred ½-3½ hours after the peak of serum Mg concentration.

The control values for peritoneal fluid Ca were 5.94-8.5 mg %. These changed relatively slightly during the experimental period, the range of maximum variation from the control values being +0.63 to −1.2 mg %, the latter occurring in the case in which the serum Ca concentration fell 4.5 mg %.

Inorganic Phosphorus. In 2 of the 3 cases in which this determination was made the serum inorganic P concentration fell from control levels of 4.6 and 6.63 mg % to minimum levels of 3.1 and 3.54 mg % respectively, 1 and 1½ hours after the peak of serum Mg concentration had been reached. A similar fall occurred in the peritoneal fluid P concentration in these cases, from control values of 4.4 and 6.36 mg % to minimum values of 3.0 and 3.6 mg %. In the other case there was no significant change in serum or peritoneal fluid P concentration.

Comment. The fall in serum Ca following injection of $MgSO_4$ is in accord with previous observations,[2, 3] some of which suggest that the SO_4 ion participates in the production of this effect. Meneghetti[4] reported a simultaneous increase in diffusible Ca (rabbits), a finding not in accord with those reported here. The observed fall in serum inorganic P has also been reported previously,[5, 6] being perhaps also dependent in part on the influence of the SO_4 ion. The simultaneous fall in diffused P (peritoneal fluid), not reported previously, is particularly significant in view of the relatively insignificant alteration in peritoneal fluid Ca concentration. It may be related to the simultaneous increase in the concentration of Mg and perhaps SO_4 in the fluid.

It is of interest that frank tetany (relieved promptly by intravenous injection of calcium glucogalactogluconate) occurred in the 3 animals in which the serum Ca concentration fell below 8 mg % despite the absence of significant alteration in the peritoneal fluid Ca concentration in 2 of these animals. The explanation of this phenomenon is not readily apparent. If, as suggested by previous reports from this laboratory,[1] the peritoneal fluid Ca concentration may be regarded as representative of the concentration of Ca in the tissue fluids, the significance of the latter in hypocalcemic tetany appears to be open to question. It is difficult, too, to understand the occurrence of tetany with relatively slight degrees of hypocalcemia in the presence of such marked increase in the Mg concentration of both serum and peritoneal fluid (and, presumably, tissue fluids).

* Aided by a grant from Parke, Davis and Co.

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Proc. Soc. Exp. Biol. Med., 45: 335-336

French Translation

The Action of Magnesium on the Excitability of the Sympathetic Nervous System

Rene Hazard and Lise Wurmser

Compt. Rend. Soc. de Biol. 108: 944-946, 1931

1. Cardiac Accelerators

The influence exerted by magnesium chloride on the excitability of these nerves in the dog is delayed following applying an induced current or adrenaline.

(a) Electrical excitation

Because of the depressing effect exerted by magnesium on cardiac rhythm in particular, after each $MgCl_2$ injection, the relative acceleration obtained must be evaluated, always together and identically, for all the excitations of the anterior branch of the Vieussens' annulus. We showed that weak doses of $MgCl_2$ (0.01 to 0.05 g $MgCl_2$/kg) given intravenously slightly increase the heart's response to the excitation of accelerators and that on the contrary strong doses (0.10 g $MgCl_2$/kg and more) tend to decrease it.

(b) Effect of adrenaline

A vagotomy or atropinization was performed on dogs in order to suppress the excitation of the vagus reflex produced by adrenaline hypertension. Under the influence of $MgCl_2$, and up to large concentrations of this salt, one sees that the same dose of adrenaline strongly and gradually increases accelerating action. This increase

due to the effect of the adrenaline asserts itself at the time when the heart's response to the electrical excitation of accelerators remains practically normal or is even diminishing.

2. Vasoconstrictors

The arterial hypertension produced by the electrical excitation of the viscera is weakened in intensity and duration by strong doses of $MgCl_2$. It is relatively more a very attenuated renal vasoconstriction, very brief particularly as related to the normal, whereas its arterial hypertension curve is still practically normal[1].

Finally we find that strong doses of $MgCl_2$ considerably lessen the hypertensive action of nicotine.

Conclusion

Whereas large doses of magnesium chloride hardly affect the electric excitability of cardiac accelerators, they increase the accelerating action of adrenaline. They exert a depressant effect on the viscera and the vasoconstrictors, especially the kidney.

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1. It is necessary to note in 2, in addition to the diminution of the renal vasoconstriction, the repression of the adrenaline excitation of the vagus reflex by $MgCl_2$.

Dog ♂ 6.6 kg; double vagotomy

Number of cardiac contractions in 10 sec.			Maximum acceleration per 100	
Without excitation	By excitation from accelerators	By action of adrenaline	By excitation from accelerators	By the action of adrenaline
--	--	--	--	--
28	37	--	32	--
28	--	35	--	25
Magnesium chloride 0.01 g/kg				
25	--	35	--	40
25	37	--	48	--
Magnesium chloride 0.01 g/kg				
18	--	30	--	66
20	29	--	45	--
Magnesium chloride 0.01 g/kg				
13	--	28	--	115
16	22	--	37	--

Compt. Rend. Soc. Biol. 108: 944-946 (1931)

ACTION DU MAGNÉSIUM SUR L'EXCITABILITÉ DU SYMPATHIQUE.

par René Hazard et Lise Wurmser.

1°. *Accélérateurs cardiaques.* — L'influence que le chlorure de magnésium exerce chez le Chien sur l'excitabilité de ces nerfs diffère suivant que l'on fait agir les courants induits ou l'adrénaline.

a) *Excitation électrique.* — En raison de l'action dépressive que le magnésium exerce particulièrement sur le rythme cardiaque, il faut, après chaque injection de $MgCl^2$, et pour toutes les excitations, toujours identiques entre elles, de la branche antérieure de l'anneau de Vieussens, évaluer l'accélération relative obtenue. On constate que les faibles doses (0,01 à 0,05 gr. $MgCl^2$ par kgr. voie intraveineuse) augmentent légèrement la réponse du cœur à l'excitation des accélérateurs et que les doses fortes (0,10 gr. $MgCl^2$ par kgr. et davantage) tendent au contraire à la diminuer.

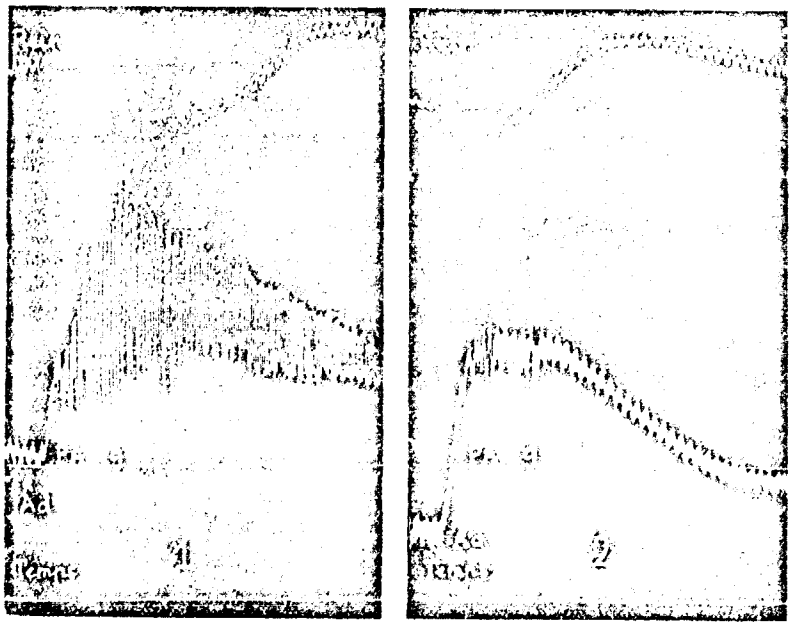
b) *Action de l'adrénaline.* — On opère chez le Chien vagotomisé ou atropinisé pour supprimer l'excitation vagale réflexe produite par l'hypertension adrénalinique. Sous l'influence de $MgCl^2$ et jusqu'à des concentrations considérables de ce sel, on voit une même dose d'adrénaline accroître fortement et progressivement son action accélératrice. Cette augmentation d'action de l'adrénaline s'affirme au moment où la réponse du cœur à l'excitation électrique des accélérateurs reste sensiblement normale ou même est en décroissance.

Chien ♂ 6,6 kgr.: vagotomie double.

Nombre de contractions cardiaques en 10 secondes			Accélération maxima p. 100	
Sans excitation	Par excitation des accélérateurs	Par action de l'adrénaline	Par excitation des accélérateurs	Par action de l'adrénaline
—	—	—	—	—
28	37		32	
28		35		25
Chlorure de magnésium 0,01 gr. par kgr.				
25		35		40
25	37		48	
Chlorure de magnésium 0,10 gr. par kgr.				
18		30		66
20	29		45	
Chlorure de magnésium 0,10 gr. par kgr.				
13		28		115
16	22		37	

2°) *Vasoconstricteurs.* — **L'hypertension artérielle produite par l'excitation électrique du splanchnique est affaiblie en intensité et en durée par les doses fortes de $MgCl^2$. Elle est relativement plus**

touchée que l'action hypertensive de l'adrénaline. De même, $MgCl^2$ affaiblit et surtout abrège la vasoconstriction rénale splanchnique ou adrénalinique. Cet affaiblissement des effets vasoconstricteurs est d'autant plus net que la dose de $MgCl^2$ injectée est plus forte. Il peut commencer à se manifester avant que $MgCl^2$ ait encore abaissé notablement la pression, ce qui permet de l'attribuer à une inhibition portée sur le sympathique



Action de $MgCl^2$ sur l'excitabilité des vasoconstricteurs par l'adrénaline. Chien ♀, 8 kgr. — Respiration artificielle, vagues intacts. Tracé oncographique du rein. Pression carotidienne avec repère à 9 cm. Hg. Temps en secondes.

En 1 comme en 2, l'animal reçoit en × Ad. 0,03 mgr. d'adrénaline en tout. Entre 1 et 2, on lui a injecté 0,10 gr. de chlorure de magnésium par kgr. par la voie intraveineuse.

vasculaire et non pas seulement à l'action dépressive et hypotensive de $MgCl^2$ qui, elle aussi, s'affirme en proportion des doses injectées ; jamais on n'observe la disparition totale de tout effet vasoconstricteur, aussi forte que soit la quantité de $MgCl^2$ que l'animal peut supporter.

L'action inhibitrice de ce sel sur le sympathique semble s'exercer assez électivement sur les vaisseaux du rein. On peut parfois constater en effet (figure ci-contre) que l'adrénaline provoque

une vasoconstriction rénale très atténuée, très brève surtout par rapport au normal, alors que sa courbe d'hypertension artérielle est encore sensiblement normale (1).

Notons enfin que les doses fortes de $MgCl^2$ atténuent considérablement l'action hypertensive de la nicotine.

Conclusion. — Le chlorure de magnésium à doses fortes, alors qu'il touche peu l'excitabilité électrique des accélérateurs cardiaques, augmente l'action accélératrice de l'adrénaline. Il exerce une action dépressive sur le splanchnique et les vasoconstricteurs, surtout ceux du rein.

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Sur la croissance résiduelle des cultures de fibroblastes,

par Boris Ephrussi et Georges Teissier.

La croissance résiduelle des cultures de fibroblastes est assez régulière et assez simple pour qu'on puisse tenter d'en donner une analyse quantitative.

Les phénomènes que nous nous proposons particulièrement d'interpréter, et qui ont déjà été signalés par l'un de nous (1*), s'observent lorsque l'on repique, dans un même flacon Carrel, deux fragments de surface inégale prélevés sur une même culture nourrie et lorsque l'on entretient ces nouvelles cultures sans leur fournir d'extrait embryonnaire.

On constate dans ces conditions que, d'une part, le rapport des surfaces maxima atteintes par les deux cultures est égal au rapport des surfaces initiales des fragments ensemencés, et que, d'autre part, la taille limite est atteinte d'autant plus rapidement que la surface originelle de la culture est plus petite. Une conséquence immédiate de ces deux faits est que le rapport de la surface de la grande culture à la surface de la petite passe par un minimum, pour reprendre à la fin de l'expérience la valeur même qu'il avait à son début.

Parmi les nombreux facteurs qui, à des titres divers, peuvent intervenir dans la croissance des cultures, deux semblent avoir, dans le cas qui nous occupe, un rôle particulièrement décisif. Le plus important est l'épuisement graduel de certaines sub-

(1) Il faut noter en 2), outre la diminution de la vasoconstriction rénale, la suppression par $MgCl^2$ de l'excitation vagale réflexe adrénalinique.

(1*) B. Ephrussi, *C. R. de l'Acad. des sc.*, 1931, t. 192, p. 1762.

The estimation of magnesium by atomic absorption spectrophotometry

A wide variety of methods have been employed for the estimation of magnesium in biological materials. All of these have suffered from the disadvantage that other substances present are liable to interfere. The principle of atomic absorption spectrophotometry, which was suggested by WALSH[1], offers greater sensitivity. DAWSON AND HEATON[2] applied this principle to the determination of magnesium. In this, light from a magnesium source is passed through a flame into which solutions containing magnesium are aspirated. Some of the resonance radiation is absorbed and the absorption of light at 285 mμ is compared with that of a standard magnesium solution treated in the same manner. This appears to give rise to a much more reliable method.

The apparatus employed by us consisted of the Hilger and Watts atomic absorption attachment which was used in conjunction with the Optica Spectrophotometer. The atomic absorption attachment can easily be aligned with the Optica; any slight adjustment in height being made with wooden blocks. The lamp housing of the Optica is moved to one side so that the atomic absorption head is in direct line with the optical system of the Optica.

Propane was used as the burner gas supply and initially the rate of flow was controlled by a two stage reduction valve. However, little or no agreement was obtained between sets of standards run on different days. This was traced to alterations in the flame concomitant with gas pressure variations. It was only when strictly controlled conditions were applied that good reproducibility was obtained. The outlet pressure on the two stage valve was maintained at 5 lb/sq. in. and the gas was then passed through a rotameter to ensure very fine adjustment. The reading on the rotameter, which was calibrated for oxygen, was set to 300 ml/min.

Similar trouble was encountered when the pressure of the air supply to the atomiser was varied. Using the strictly controlled propane flow it was found that alterations in air pressure greatly affected the readings obtained for standard solutions of magnesium. The control of the air pressure was therefore brought about by a two stage reduction valve and the optimum air pressure was found, by trial and error, to be 20 lb/sq.in. It should be pointed out that it was necessary to alter the height of the burner each time that any change was made in the air or gas supplies to ensure that the light from the magnesium lamp passed through the optimum part of the flame. Once the optimum condition of flow rates for air and propane had been determined no further alterations were required in the burner height or the rates of flow of gas.

Standard solutions of magnesium were made up using magnesium sulphate ($MgSO_4 . 7H_2O$; Analar). An initial stock solution containing 10 mg Mg/100 ml (8.3 mequiv. Mg/l) was used and this was diluted to give a solution of 1 mg Mg/100 ml (0.83 mequiv. Mg/l). Dilutions to cover the range 0.01 to 0.10 mg Mg/100 ml (0.008 to 0.08 mequiv. Mg/l) were prepared immediately before use.

The solutions used were acidified with hydrochloric acid as suggested by DAWSON AND HEATON[2]. At the recommended gas flow rates for the estimation, alteration of the hydrochloric acid concentration of the solutions over a wide range, namely from approximately 0.1 *N* to 1.2 *N*, did not affect the results obtained either with the

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The Estimation of Magnesium in Plasma, Muscle and Bone, by Atomic Absorption Spectrophotometry

B. J. Hunt

The use of the EEL 140 atomic absorption spectrophotometer for the determination of magnesium in plasma, skeletal muscle, and bone is described together with an examination of the pattern of chemical interference in each type of sample. The sampling procedure and preparation of each kind of sample for analysis is detailed, and recovery studies on plasma bone and muscle are presented. The mean value for plasma magnesium in 42 normal men and women was 2.16 mg/100 ml ± SD of 0.08. For 30 male Wistar strain rats, the plasma value was 2.10 mg/100 ml ± SD of 0.18. In 15 normal postmortem human muscle samples, the mean magnesium content was found to be 93 mg/100 g of dry muscle, the series range being between 83 and 101 mg/100 g. The comparable value in 49 male rats was 125 mg/100 g dry weight ± SD 1.8. Bone magnesium in femur diaphysis of 20 male rats (average weight 200 g) was 563 mg/100 g dry weight ± SD 12.7. It is concluded that this instrument, when operated in the manner suggested in our study, is technically easy to use, and gives precise, sensitive, and reproducible results for magnesium in a variety of biologic material.

THE TECHNICAL DIFFICULTY of measuring magnesium in biologic material has been the main source of the obscurity which still shrouds magnesium metabolism studies in the human and animal organism. Since Walsh's original publication in 1955 (*1*), however, the use of atomic absorption spectrophotometry (AAS) for the determination of magnesium has received wide acclaim (*2–7*).

This paper describes our experience with the EEL 140 (Evans Electroselenium Ltd) atomic absorption spectrophotometer, which was used to determine magnesium in plasma, skeletal muscle, and bone. Studies designed to detect the pattern of chemical interferences by other ions in each type of sample are included, together with sampling technics and recovery studies. Normal values for human and rat plasma

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and skeletal muscle magnesium, and rat bone magnesium, are also presented.

Materials and Methods

Apparatus and Operating Procedure

The theoretic considerations underlying the operation of the EEL 140 are the same as for any absorption spectrophotometer and, as such, have been described elsewhere (*8–12*). The EEL 140 is one of the smallest and least expensive spectrophotometers presently on the market and can be operated by technicians with a minimum of training. Experience has taught us to observe the following modifications of the technic set out in the instruction manual for operation at maximum sensitivity:

1. At least 40 min should be allowed for lamp warming before starting operations. The burner should be lit for the last 10 min of this time, and water at room temperature should be aspirated for the last 5 min. This last procedure maintains a constant operational temperature in the aspiration channels and prevents falsely high values from occurring in the first few standards from a temperature-dependent increased aspiration rate.

2. A small air compressor, which also filters and dries the air, is preferable to cylinders of compressed air. The latter are economically disadvantageous. Dry air is essential in order to prevent variations in reading due to seasonal changes in humidity.

3. Fine adjustment of the burner is mechanically impossible. It has been proved impracticable, therefore, to use the metric scale for the "direct readout" measurement of concentration as suggested in the manual. The burner should be rotated to obtain the maximum forward deflection for any given standard and not moved again during the session of analysis.

4. Maximum sensitivity is achieved by tuning into the peak of the wave length as described in the manual. The wave length registered at the peak absorption may be anywhere from 2830 Å to 2875 Å, although the magnesium wave length is 2852 Å. The importance of operating always at the peak of the wave length is illustrated by Fig 1. This shows an absorption spectrum of a standard magnesium solution containing 0.8 ppm (parts per million; 0.8 μg/ml) over wave lengths ranging between 2400 Å and 3300 Å. The peak of the absorption curve is needle sharp and occurs, in this instance, at 2848 Å. It clearly can be seen that reading a fraction away from the peak will markedly decrease sensitivity—eg, if the sample were read at 2850 Å, a deviation of under 0.1% exists, yet sensitivity will decrease by 25%. Thus,

retuning should be carried out several times during a normal session to assure the maintenance of maximum sensitivity.

5. The aspiration rate must also be maintained constant for operation at full sensitivity. The actual aspiration rate depends again on

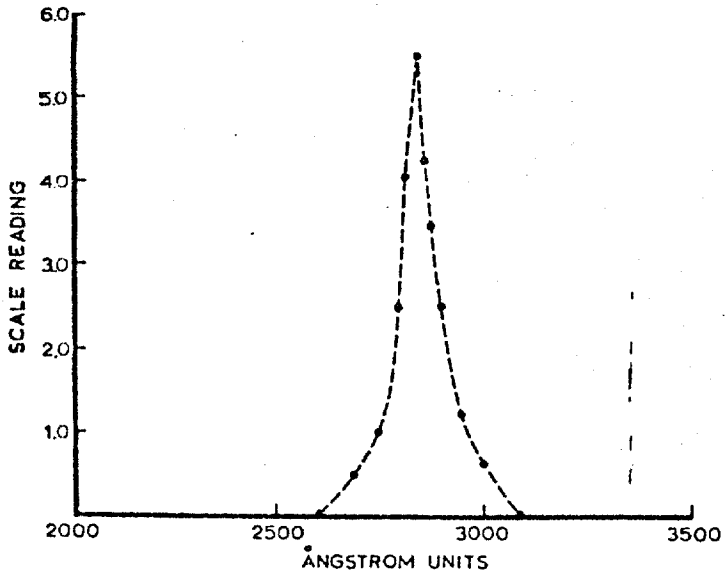

Fig 1. Absorption curve of a reference standard containing 0.8 ppm of magnesium.

individual aspirators but usually lies between 6 and 9 ml/min. We have found repeatedly that the major source of reduction in sensitivity is nearly always due to a blocked or faulty aspirator.

6. Both the slit width and air/acetylene pressure should be adjusted to obtain the maximum forward deflection of the meter needle for any given standard. Exact values will vary according to the instrument and even in the same instrument following part replacement.

Reagents and Preparation of Standards

Treatment of Water

Fresh glass-distilled water which had been passed through a mixed cation exchange resin bed was used for the final rinses of all glass and plastic (Nalgene) ware. For the preparation of all standard solutions, a further distillation in Yoe's glass distillation apparatus was carried out on the resin-treated water. This water was designated as "top quality water" and contained no detectable calcium, but 0.01 mEq/L of magnesium, which value remained unchanged throughout further distillation.

Storage of Samples

Nalgene ware was used exclusively in the preparation and storage of all samples and solutions. In cases where the use of glass apparatus was essential, for example, the use of volumetric flasks or glass test tubes for separation of plasma from whole blood etc., transfer of samples to Nalgene containers was carried out promptly, so that minimal contact with glass was assured.

Whole plasma samples were stored in the refrigerator at 2° if analysis was to take place within 24 hr of collection, otherwise, samples were frozen. No loss of magnesium was found to occur up to 12 months (maximum time observed) in these samples. However, diluted plasma, even at a dilution of 1:2, when frozen and stored, showed a gradual loss of up to 25% of magnesium over a 6-month period (see Discussion).

The magnesium content of muscle and bone remained unchanged up to 12 months when frozen, and concentrated solutions of both samples will also keep satisfactorily if frozen—eg, the original 10 ml which contains the sample after ashing. Weaker dilutions show a gradual reduction in magnesium content as seen in the diluted plasma.

Magnesium Standard Solutions

1. Preparation of Stock Standard Solution from Magnesium Metal Rod

A solution containing 1000 ppm of magnesium (1000 μg/ml) was made from magnesium metal rod as follows. The metal was scraped free of magnesium oxide until shining, at which time a portion was sawed off. Exactly 1.00037 g (magnesium rod is 99.963% pure magnesium) is weighed out in a tared weighing vessel and quantitatively transferred to a 1-L flask with 2 ml of top quality water. Five milliliters of 5 N redistilled HCl are then added, which is sufficient to dissolve all the magnesium metal present. When cool, the solution is made to volume with top quality water and transferred to a Nalgene airtight container and stored at 2°.

2. Preparation of Magnesium Standards from a Magnesium Reference Solution

Detailed comparative tests between the concentrated standard solution prepared from magnesium metal as above and an atomic absorption reference solution (Fisher Scientific Company) of 1000 ppm (1000 μg/100 ml) of magnesium ion gave identical results in all respects. This commercial standard solution, therefore, is now used routinely for preparation of all magnesium standard solutions.

Dilution of the reference standard is carried out as follows: 10 ml of the concentrated stock solution is diluted to 100 ml with top quality water, and 10 ml of this solution is further diluted to 100 ml. This re-

sults in a final solution of 10 ppm (10 μg/ml). Working standards containing 0.2–0.8 ppm (0.2–0.8 μg/ml) are made by diluting 2, 3, 4, 5, 6, 7, and 8 ml of the 10 ppm solution to 100 ml. Although the 10 ppm solution can be obtained by diluting 1 ml of the stock standard in 100 ml of water, we found the 2-step preparation of the 10 ppm solution and the initial use of a relatively large volume (10 ml of original stock solution) gave results so consistent that the standard curve does not change from month to month. Working standards are prepared by direct dilution of the concentrated 1000 ppm solution, and the intermediate dilutions are discarded. The standards are prepared freshly every 3 weeks, or when the level of fluid in the 125 ml Nalgene container measures an inch or less.

The standard curve routinely used contains magnesium 0.2–0.8 ppm, in water or 0.5% (w/v) lanthanum chloride, depending on the sample to be analyzed (see below). A straight line was obtained over this concentration range in both diluents. Three typical standard curves of magnesium in water and magnesium in 0.5% $LaCl_3$, with and without HCl, are shown in Fig 2.

3. Preparation of Stock $LaCl_3$ Solution

Exactly 144.052 g of $LaCl_3$ (purified, Fisher Scientific Company) is weighed, placed in a 2-L volumetric flask and made to volume with top

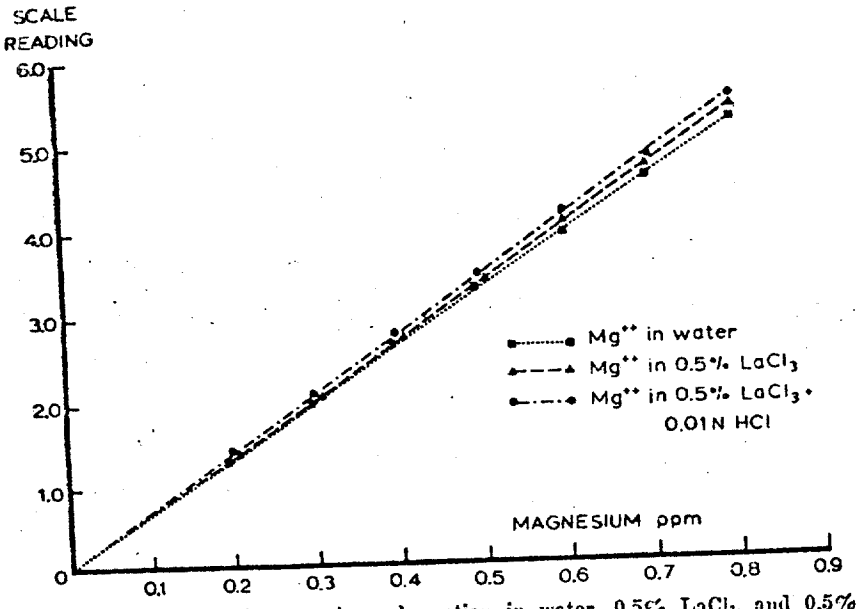

Fig 2. Standard curves of magnesium absorption in water, 0.5% $LaCl_3$, and 0.5% $LaCl_3$ with 0.01 N HCl.

quality water. This 5% stock solution is then allowed to remain at room temperature overnight and is then filtered through fine chemically clean glass wool and stored in a Nalgene container at 0.2°. Ten milliliters of this solution is added to each 100 ml of tissue and bone working standards so that the final concentration of Lanthanum is 0.5%.

4. Preparation of Stock Electrolyte Solutions for Use in Chemical Interference Studies

Calcium Reference Solution 10,000 ppm (10 mg/ml) (Fisher Scientific Company) This solution was diluted so that the working standard contained 100 ppm (100 μg/ml).

Primary Sodium Phosphate (NaH_2PO_4 H_2O) Reagent Grade (Fisher Scientific Company) 26.7352 g of this salt made up to volume in 1 L of top quality water gives a solution containing 6000 ppm (6000 μg/ml) of inorganic phosphate.

Potassium Chloride (KCl) Reagent Grade (Fisher Scientific Company) Stock solution contained 1000 ppm (1000 μg/ml) of K; this was obtained by dissolving 1.90702 g of the salt and making up to volume in 1 L of top quality water.

Sodium Chloride (NaCl) Reagent Grade (Fisher Scientific Company) A stock solution containing 1000 ppm (1000 μg/ml) was made by dissolving 2.54237 g of salt in 1 L of top quality water.

Protein Two preparations of human albumin were used for these studies: (1) 25% (w/v) of normal serum albumin contained in a salt-poor, buffered solution (Connaught Medical Research Laboratories, Toronto), and (2) human albumin powder, Grade 3 (98% pure) Sigma Chemical Company. Stock solutions containing 100 mg/ml were made from each preparation. No difference on magnesium absorption was observed between the two solutions.

These electrolyte and protein solutions were diluted to obtain the varying ratios (measured as μg/ml of salt to magnesium required as shown in Table 2 (see below).

Preliminary Studies

1. Detection of Chemical Interferences

The work of this laboratory is concerned with the measurement of magnesium in a variety of biologic material, of both human and animal origin. The main purpose of the preliminary experiments, therefore, was to detect possible chemical interference by major electrolytes present in each kind of sample to be analyzed. For this purpose, rat and human values of all the major electrolytes—sodium, potassium, calcium, phosphate, and magnesium—in serum, muscle, and bone were obtained from the literature (Table 1), and the maximum ratio of each

standard solutions or with sera. For the estimation, 0.1 ml of serum or standard was pipetted into approximately 4 ml of distilled water, 1 ml of concentrated hydrochloric acid was added and the volume made up to 10 ml with distilled water.

The various standard curves obtained with different flow rates of propane and different air pressures are shown in Fig. 1; the marked effect of the pressures on the results obtained is evident.

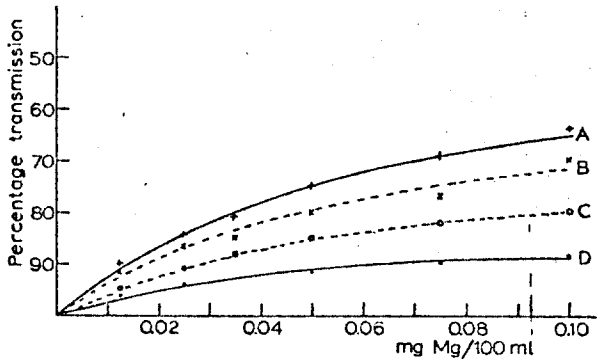

Fig. 1. Variation in standard curve using different flow rates of gas. (A) 20 lb/sq.in. air and 300 ml/min propane; (B) 15 lb/sq.in. air and 300 ml/min propane; (C) 15 lb/sq.in. air and 250 ml/min propane; (D) 20 lb/sq.in. air and 250 ml/min propane.

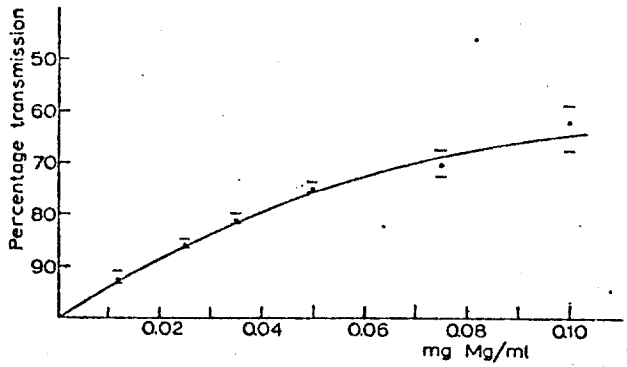

Fig. 2. Mean standard curve obtained. The horizontal bars indicate the upper and lower values of 40 different estimations at each point.

Fig. 2 shows the variation obtained when the standard curves were set up and determined on forty different occasions. The continuous line is the mean value of all the readings and the horizontal bars indicate the maximum and minimum values obtained at each of the points. The standard deviation of the results obtained when a serum sample, containing 1.8 mequiv. Mg/l, was set up and determined forty times was $\pm$ 2%.

The main sources of interference in existing methods of estimating magnesium are protein, sodium, potassium, calcium, phosphate and chloride ions and the possible effects of these substances on the present method were investigated.

The addition of albumin to the standard solutions to give final protein concentrations ranging between 0.1 mg albumin/ml and 2.0 mg albumin/ml did not affect

the readings obtained. Working on the basis of a 1 in 50 dilution for serum, the above protein solutions would be equivalent to initial serum albumin concentrations of 0.5–10.0 g/100 ml. This range is much wider than that likely to be met in clinical conditions. The only obvious effect of the addition of the albumin was the more uniform wetting of the spray chamber.

Sodium chloride was added to the aqueous standards in concentrations ranging from 2 mequiv. Na/l to 3.5 mequiv. Na/l before the magnesium was determined. These sodium concentrations, allowing for an initial 1:50 dilution for serum, are equivalent to 100–175 mequiv. Na/l. The sodium present did not interfere with readings obtained. Similar results were obtained when potassium chloride was added to give final concentration of 0.03 mequiv. K/l to 0.15 mequiv. K/l (*i.e.* equivalent to serum potassium concentration of 1.5–7.5 mequiv. K/l).

The addition to the aqueous standards of calcium chloride in amounts equivalent to initial serum concentrations of 2.5–7.5 mequiv. Ca/l similarly did not alter the results.

By the addition of phosphoric acid to the standard samples it was shown that the concentrations of phosphate likely to be encountered would not affect the results.

When serum samples were de-ionised by passage through an ion exchange column (cation exchanger I.R. 120) no readings were obtained by our standard procedure. Magnesium sulphate, varying in amounts from 0.008–0.08 mequiv. Mg/l, was added to 20 samples of de-ionised serum and the recovery of the added magnesium was found to be 98 ± 2%.

The recovery of magnesium when added in similar amounts to 20 serum samples was found to be 96 ± 2%.

Similar findings were obtained with de-ionised and also untreated urine.

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A manifold for drying organic solvent extracts under reduced pressure

Evaporation of organic solvents from multiple extracts of biological materials is very tedious and time consuming. To facilitate this procedure a simple manifold handling up to 6 samples simultaneously has been developed in our laboratory.

Evaporation is carried out under reduced pressure. A valve controlled air or gas leak ensures even evaporation and prevents accidental losses of the extract by spurting.

Clin. Chim. Acta, 8 (1963) 976–978

Table 1. Maximum Average Electrolyte Values for Serum, Skeletal Muscle, and Bone*

Material	Mg^{++}	Na^{+}	K^{+}	$PO_4^{=}$	Ca^{++}
Serum (mg/100 ml)	2	320	20	4	10
Skeletal muscle (mg/100 g wet weight)	24	85	420	240	8
Compact bone (mg/100 g dry weight)	520	700	312	13,000	30,000

* These figures are approximate and represent both human and rat values. The main source of these data is Widdowson, E. M., and Dickerson, J. W. T. "Chemical Composition of the Body" In *Mineral Metabolism*, Vol. 2A. C. L. Comar and F. Bronner, Eds. New York: Acad. Press, 1964.

Table 2. Ratio of Electrolytes to Magnesium in Serum, Skeletal, Muscle and Bone

Source	Mg^{++}	Na^{+}	K^{+}	$PO_4^{=}$	Ca^{++}	Protein
Serum	1	*160*	10	2	5	4000
Skeletal muscle	1	4	*18*	10	< 1	*
Bone	1	1.3	< 1	*26*	*60*	*

* Not present in final preparation.

ion to magnesium was calculated on a microgram basis (Table 2). In addition, two concentrations of the purified plasma human albumin were also investigated in concentrations of 8 g/100 ml and 4 g/100 ml of plasma.

Interference studies were carried out by observing the effect of each individual ion when present in the standard solution in ratios up to and exceeding the maximum ratio observed in any particular tissue. For example, to observe the effect of phosphate on magnesium absorption, solutions containing phosphate in ratios to magnesium of 2:1, 10:1, and 50:1 were added to magnesium standards containing 0.2–0.8 ppm (0.2–0.8 μg/ml) and the samples were read against a standard curve containing magnesium 0.2–0.8 ppm in water. Similar studies were carried out with calcium, sodium, potassium, and protein. The results of the studies are shown in Table 3.

It is seen that calcium, even when present in a concentration ×100 of that of magnesium, has no effect on magnesium absorption, while phosphate increases magnesium absorption even when present in a ratio of 1:2. This effect increases when phosphate is present over a ratio of 1:20. Sodium has only slight effects on the absorption of magnesium, and this occurs only when sodium is present in a ratio over 1:100, as would be found in serum. Similar findings are seen with potassium, although in this case potassium must be present in con-

Table 3. Effect of Single Electrolytes on Magnesium Absorption

Ratio to Mg	Ca	P (%)	Na (%)	K (%)	Pr (albumin)* Apparent (%)	Pr (albumin)* Real (%)
1:1	NE	—	—	NE	—	—
1:2	—	+ 7.5	—	—	—	—
1:5	NE	—	NE	—	—	—
1:10	—	+ 7.5	—	NE	—	—
1:20	—	+ 7.5	NE	NE	—	—
1:50	NE	+10	—	—	—	—
1:100	NE	—	+2.5	+2.5	—	—
1:200	—	—	+2.5	—	—	—
1:2000	—	—	—	—	+ 9.5	< 2
1:4000	—	—	—	—	+19.0	< 2

Figures refer to % change in absorption compared with standard magnesium solution containing 0.4 ppm (0.4 μg/ml); NE = no effect.
* See text.

centrations exceeding its content in serum, skeletal muscle, and bone, the figure of 1:100 being related more closely to the ratio of potassium to magnesium in red blood cells. Maximum deviation was found when protein was added to magnesium standard showing an enhancement directly proportional to the concentration of protein in the solution. This finding is discussed in detail below.

2. The Effect of Suppressant Agents

The interference studies were repeated in the presence of two suppressant agents at two strengths, namely, strontium chloride 0.5% and 1% and lanthanum chloride 0.5% and 1%. Marked fluctuation in values was observed when $SrCl_2$ of both concentrations was used, and no consistent pattern emerged during the interference studies. The main reason for this was found to be clogging of the burner which took place with even the 0.5% solution after 5–10 min operation of the instrument. Lanthanum chloride, however, completely suppressed the enhanced absorption of magnesium seen in the presence of potassium, sodium, and phosphate in all bone and soft tissue solutions. The use of $SrCl_2$, therefore, was abandoned, and $LaCl_3$ in a final concentration of 0.5% was subsequently used in the magnesium determinations of both muscle and bone samples. Neither suppressant, however, had any effect on the considerable enhancement of magnesium absorption by protein. Previous experiments in which plasma protein had been precipitated by 10% trichloroacetic acid had shown not only a loss of up to 20% of magnesium in the protein-free filtrate, but also values which varied greatly with degree of dilution (see Fig 3). Removal of plasma protein, therefore, did not appear to be the ideal solution to

the problem. Fortunately, further examination of the two commercial albumin preparations showed that they were both heavily contaminated with magnesium, which amounted to 0.6 mg/100 ml in an 8% solution. The actual enhancement of magnesium absorption by protein was

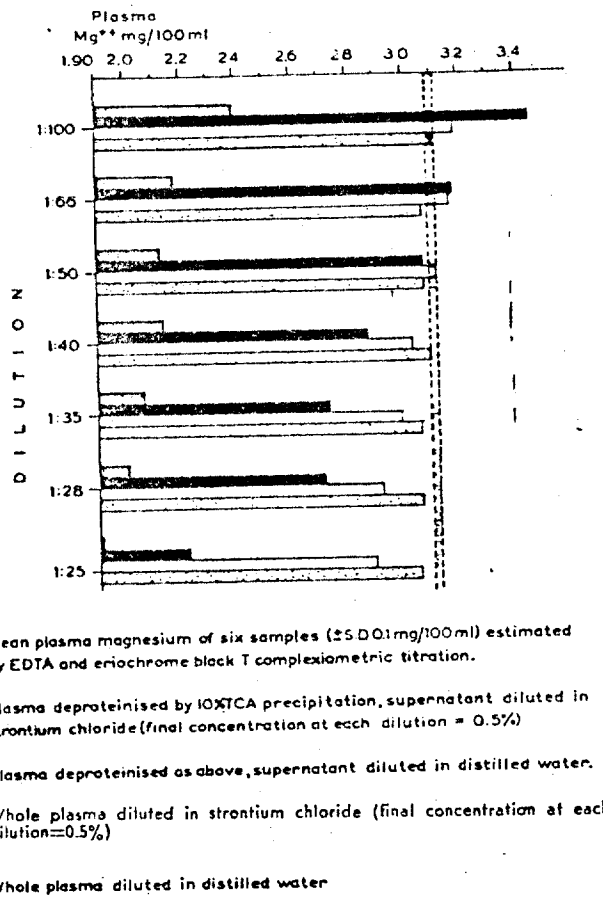

Fig 3. Magnesium concentration of pooled plasma estimated in the presence and absence of protein and strontium chloride, at various dilutions.

found to be under 2% when correction was made for this artifact. Subsequent studies were carried out, therefore, on whole plasma and diluted distilled water.

The effects of dilution and sample size on the precision of plasma magnesium estimations were investigatel as follows: Plasma samples ranging between 0.1 and 4.0 ml were diluted by factors varying from 10 to 100 with top quality distilled water. Each dilution and reading was done in duplicate. Results showed that the maximum variation between duplicate and/or samples was under 1%. Thus, precise and

reproducible results can be expected when samples ranging between 0.1 and 4.0 ml are diluted by a factor of up to 1:100. In terms of the actual concentration of magnesium in the sample, this represents 0.2–0.8 μg/ml. In practice, a sample size of 0.2 ml of plasma is used, which is made to volume in a 10 ml volumetric flask by top quality water.

Estimation of Plasma Magnesium

1. Sampling Technic; Human Subjects

Approximately 5 ml of venous blood was taken without tourniquet from the antecubital fossa of normal subjects (healthy students and laboratory personnel) with a dry, chemically cleaned syringe (the skin was allowed to dry thoroughly after alcohol application before venesection). The needle was removed from the syringe, and the blood placed in a glass test tube coated with dry Heparin which had previously been contained in 3 drops of a 1% heparin solution. The blood was centrifuged within 20 min of venesection, and the plasma was separated and placed in polyethylene test tubes. If samples were to be estimated within 24 hr, the test tubes were stored in the refrigerator, otherwise storage was carried out in the freezer at −20°.

Rat Samples

Values reported here for rat plasma magnesium were obtained by resection of the abdominal aorta of rat under ether anesthesia. The sample volume varied from 5 to 10 ml of whole blood, depending upon the size of the rat. Blood was collected into glass test tubes coated with dried heparin equivalent to 3 drops of a 10% solution. It is of interest to note, that in order to anticoagulate the same volume of rat blood as human, heparin 10 times the concentration of that used in humans was required.

2. Recovery Studies

One milliliter of a standard solution was added to each of four 1 ml aliquots of pooled plasma, and contained one of the following concentrations of magnesium (as magnesium chloride): 20 μg/ml, 10 μg/ml, 5 μg/ml, and 2.5 μg/ml. Each plasma aliquot with its magnesium salt addition was diluted to 100 ml and read against the standard curve of magnesium from 0.2 to 0.8 ppm in water. The results, as shown in Table 4 demonstrate a recovery rate of 99.5%.

3. Plasma Magnesium Values in Normal Humans and Rats

Plasma magnesium in 42 normal men and women was 2.16 mg/100 ml ± SD 0.09 mg (Table 5) and in 30 normal Wistar strain rats, 2.10 mg/100 ml ± SD 0.18 mg (Table 6).

Table 4. Recovery Studies on Plasma Magnesium

Samples	Mg^{++} added (μg/ml)	Mg^{++} expected (mg/100 ml)	Mg^{++} estimated (mg/100 ml)	% Recovery
Control (×3)	—	—	2.089	—
1	2.5	2.339	2.331	99.7
2	5.0	2.589	2.572	99.3
3	10	3.089	3.086	99.9
4	20	4.089	4.050	99.1
Mean recovery				99.5

Estimation of Skeletal Muscle Magnesium

1. Sampling Technic

Immediately following death by exsanguination from the abdominal aorta under ether anesthesia, muscle samples between 700 and 900 mg were dissected from the ventral thigh of normal rats. The samples were carefully but rapidly dissected free of the sarcolema, ligaments, and other extraneous matter, weighed on a torsion balance, and placed in a small chemically clean, airtight, polyethylene vial. These were placed in the freezer within 5 min of sampling. The most suitable muscle for electrolyte studies in the rat was found to be the adductor magnus which is situated immediately under the gracilis muscle. This muscle is usually entirely clear of fat and has only one small tendon of insertion and one nerve, which are easily removed.

2. Preparation of Sample for Analysis

On the day of estimation, the sample was removed from the freezer and allowed to reach room temperature. It was then quantitatively transferred to a tared platinum crucible with approximately 1 ml of top quality water, dried at 90° for 16 hr in a gravity oven, and for a further 12 hr in a vacuum oven at the same temperature. The samples were then placed in a dessicator to cool and weighed after a minimum of 4 hr had elapsed. Dry ashing was carried out for 16 hr in a muffle furnace, at a temperature of 600°. After cooling, 2 ml of 5 N redistilled HCl was added to each sample, and the crucibles were then placed in a water bath for 30 min. This procedure resulted in a completely clear pale yellow solution which was then quantitatively transferred to 10 ml volumetric flasks with hot top quality water. The samples were made to volume when the solution had reached room temperature.

As our preliminary studies had shown that 0.5% $LaCl_3$ was sufficient to suppress any interference by any other ions present in the sample, the magnesium standards for tissue magnesium were made to contain 0.2–0.8 ppm (0.2–0.8 μg/ml) of magnesium, each in a final dilution of 0.5% $LaCl_3$. In addition, each standard also contained 0.01 N HCl in

Table 5. Human Plasma/Serum Magnesium Values Estimated by AAS from Literature

Study No.	*Ref. No.*	*Year*	*No. estimations*	*Plasma*	*Serum*	*Dilution factor*	*Treatment of protein*	*Diluent*	*Magnesium (mg/100 ml)*	*SD ± mg/100 ml*
1	4	1963	100	—	+	1:20	TCA ppt*	Water	2.12	0.13
2	13	1964	10	—	+	1:50	None	Water	2.60	0.21
3	14	1965	33	+	—	1:11	None	$SrCl_2$	1.92	0.14
4	15	1966	80	—	+	1:10	TCA ppt	Water	1.96	0.10
5	16	1966	54	—	+	1:26	None	$SrCl_2$	2.06	0.13
6	17	1967	24	—	+	1:17	Dialysis (automated)	$LaCl_3$	2.14	0.24
7	Present series	1968	42	+	—	1:100	None	Water	2.16	.08

* Trichloracetic acid precipitation.

Table 6. Magnesium Content of Plasma, Skeletal Muscle, and Bone in the Male Rat

Source	No. of Samples	mg/100 ml ± SD	wet wt (mg/100 g ± SD)	dry wt (mg/100 g ± SD)
Plasma	30	2.10 ± 0.18	—	—
Skeletal muscle	49	—	30.0 ± 0.43	125 ± 1.8
Bone	20	—	484 ± 11.2	563 ± 12.7

the final dilution, which concentration corresponds to that used for dissolving the ash.

The dilution required for muscle weighing in the region of 750 mg is 1:1000, and the final solution also contains 0.5% $LaCl_3$. Thus, in practice, 1 ml of the original 10 ml solution and 10 ml of a 5% $LaCl_3$ is placed in a 100 ml volumetric flask and made up to volume with double distilled water.

3. Recovery Studies

Table 7 shows the result of the recovery studies when magnesium (as magnesium chloride solution) is added in concentrations ranging between 60 μg and 180 μg to dry homogenized pooled muscle samples ranging in weight between 50 mg and 200 mg. The mean recovery rate was 99.27%.

A further study to test the reproducibility of both the sampling procedure and the analytic technic was carried out by analyzing left and right adductor magnus muscle in 10 male Wistar strain rats weighing between 250 and 320 g. These results calculated on a dry and wet weight basis are shown in Table 8. It may be noted that these values, which represent normal skeletal muscle magnesium content, are considerably higher than those values shown in Table 7. The reason for this is that the tissue fluid which was released during the homogenizing procedure was discarded so that the magnesium content of the muscle used during the recovery studies was deliberately an 'unknown.'

4. Normal Human Skeletal Muscle Magnesium

The mean magnesium content of 15 human skeletal muscle samples was 76.3 mEq/kg dry wt. This value compares favorably with the available published data (Table 9). The samples were obtained from the pectoralis major muscle from 1–6 hr following death from trauma. The ages of subjects ranged between 13 and 80 years.

5. Normal Rat Skeletal Muscle Magnesium

The magnesium content of 49 male Wistar strain rats ranging in weight between 250 and 550 g was 30.07 mg/100 g wet weight ± SD 0.43 (Table 6).

Table 7. Recovery Studies on Muscle Magnesium

Sample No.	Dry wt (g)	Magnesium (μg) Estimated	Calculated	Added	Expected	Estimated	Recovery (%)
			CONTROLS				
1	.19443	148.9	—	—	—	—	—
2	.19485	148.9	—	—	—	—	—
3	.19587	150.0	—	—	—	—	—
4	.19631	150.0	—	—	—	—	—
			EXPERIMENTS				
5	.04939	—	37.78	180	217.78	215.37	98.89
6	.04990	—	38.17	180	218.17	215.37	98.72
7	.19664	—	150.43	120	270.43	270.32	99.96
8	.19800	—	151.47	120	271.47	270.32	99.58
9	.09721	—	74.37	120	194.37	193.09	99.34
10	.09786	—	74.86	120	194.86	194.57	99.85
11	.14510	—	111.00	60 μg	171.00	169.32	99.02
12	.14553	—	111.33	60 μg	171.33	169.32	98.83
Mean recovery							99.27

Table 8. Comparison of Magnesium in Paired Samples of Rat Skeletal Muscle

Sample No.	Mg/100 g wet weight R thigh	L thigh	Mg/100 g dry weight R thigh	L thigh
1	30.10	29.71	119.3	122.3
2	28.87	29.23	121.9	124.1
3	29.08	30.12	121.7	126.6
4	29.25	29.34	123.6	124.0
5	30.42	30.35	126.5	126.9
6	30.47	30.59	125.9	125.1
7	29.14	30.27	121.6	124.6
8	29.53	30.63	126.1	126.9
9	29.10	30.17	123.7	126.5
10	29.06	30.13	123.9	124.9
11	29.00	28.82	122.2	119.5
12	29.18	29.67	120.9	123.3

Table 9. Human Skeletal Muscle Magnesium

Authors	No. of estimations	Mean value Mg^{++} (mg/100 g dry muscle)	Series range
Dickerson & Widdowson (*18*)	3†	98	81–102
	3‡	101	90–116
MacIntyre *et al* (*19*)	5	+ 86.0*§	77–95
Present series	15	93	83–101

* Calculated from author's data.
† Minimal hemorrhage before sampling.
‡ Severe hemorrhage before sampling.
§ Estimated on dry fat free muscle.

Estimation of Bone Magnesium

1. Sampling Technic

The midshaft of the right femur in each rat was isolated and scraped completely free of muscle, periosteum, and ligaments with a sharp scalpel. Each bone was then split longitudinally and opened using micro bone cutters—a procedure which usually produced three fragments. The marrow was removed from each fragment by scraping with a small scalpel blade. Subsequently, each fragment was cleaned with a gauze swab slightly moistened with 0.9% sodium chloride. At the end of this procedure, the bone was white and shining and completely free of blood. The fragments were wiped dry and then immersed in ether for approximately 10 min, following which they were removed, wiped, and allowed to stand at room temperature for 10 min to complete ether evaporation. This procedure was instituted in order to obtain an accurate wet weight of bone and does not constitute a procedure for fat extraction. When thoroughly dry, exact weights were obtained on the torsion balance, with the samples usually weighing between 110 and 120 mg. The fragments were then placed in a chemically clean small Nalgene container and frozen at −20° until the time of analysis.

2. Preparation

Bone samples were transferred to platinum crucibles, dried to constant weight, cooled, and the dry weight taken as described for muscle. The bone was ashed in the muffle furnace for 16 hr at 600°, followed by 4 hr at 800°. This procedure insured complete ashing without reduction in electrolyte values which occurs if high temperatures are maintained for a longer period of time. Subsequent dissolving of ash and transference to 10-ml volumetric flasks was carried out as described for muscle.

Routinely, normal bone of approximately 100–130 mg is diluted 2000 times (0.5 ml of the original 1:10 dilution, in 100 ml of double distilled water). The final dilution contained 0.5% $LaCl_3$ and 0.005 N HCl. Samples were read against a standard curve containing magnesium 0.2–0.8 ppm (0.2–0.8 μg/ml), 0.5% $LaCl_3$ and 0.005 N HCl, as was present in the sample.

3. Recovery Studies

Recovery studies were carried out on pooled dried rat femur, which had been ground to a fine powder. Aliquots of bone weighing 20–100 mg were taken, and magnesium (as magnesium chloride solution) was added in quantities from 25 to 400 μg. Each sample was then dried, ashed, and treated as already described. The results of these studies,

Table 10. Recovery Studies on Bone Magnesium

Sample No.	Dry wt (mg)	Magnesium (μg) Estimated	Calculated	Added	Expected	Estimated	Recovery (%)
			CONTROLS				
1	100	460.1	—	—	—	—	—
2	100	460.1	—	—	—	—	—
3	100	460.1	—	—	—	—	—
4	100	460.1	—	—	—	—	—
			EXPERIMENTS				
5	20	—	92.02	400	492.02	491.34	99.87
6	20	—	92.02	400	492.02	491.34	99.87
7	100	—	460.1	250	710.10	710.00	99.90
8	100	—	460.1	250	710.10	710.00	99.90
9	50	—	230.05	250	480.05	480.00	99.90
10	50	—	230.05	250	480.05	480.00	99.90
11	80	—	368.08	100	468.08	468.60	100.10
12	80	—	368.08	100	468.08	468.60	100.10
13	100	—	460.1	25	485.10	478.00	98.54
14	100	—	460.1	25	485.10	475.00	98.00
Mean recovery							99.61

seen in Table 10, show a mean recovery rate of 99.61%. Mean values for 20 normal rat femur diaphysis was found to be 563 mg/100 gm dry weight ± SD 12.7 (Table 6). This value compares well with those of Hammet (*20*) and Smith and Field (*21*) whose calculated data give values of 540 and 520 mg/100 g dry bone, respectively.

Discussion

Methods for the measurement of blood, muscle, and bone magnesium have been described in detail. Following the measures indicated, reproducible results have been obtained in a variety of biologic materials as well as excellent recovery of added known amounts of magnesium.

Some loss of Mg^{++} occurs upon standing under refrigeration in dilute solutions even if stored in Nalgene ware. It is possible that the inside of the container develops a negative electrostatic charge which firmly binds magnesium to the surface. Alternately, the solution may move into pores of the polyethylene by capillarity, and again electrostatic charges developing around the mouth of the pore might trap ions inside. Whatever the cause, only the most concentrated standard solution of standard or sample should be stored for any length of time. This phenomenon has also been observed with calcium.

The use of platinum crucible was found to be important. The Vycor

brand crucible (silica) and quartz crucibles gave inconsistent and grossly abnormal recovery values for both magnesium and calcium. Both types of crucibles developed a permanent white opaque film at the bottom which could not be removed with concentrated acid. This could represent insoluble calcium phosphate complex as observed by other investigators (*4*).

The reason for the variability of normal plasma magnesium values (Table 5) is probably found in the diversity of instruments and component parts used in each study as suggested by Stewart and Fleming (*15*). It seems, therefore, that a method for producing a confident, well defined narrow range as reflected by a small standard deviation is more important than the pursuit of an absolute serum magnesium value.

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A General Method for Magnesium Analysis in Biological Materials by Atomic Absorption Spectroscopy[1]

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Since the advent of atomic absorption spectroscopy a number of methods for the measurement of magnesium in biological materials have been developed to exploit the potential of this sensitive procedure. However, a concerted effort to establish a general method applicable for magnesium analysis in a wide variety of biological samples has not been undertaken. To achieve such a method it is necessary to examine a number of parameters which influence the precision and accuracy of magnesium analysis by atomic absorption spectroscopy. These include cation and anion interferences, sample treatment, the properties of the burner, and the characteristics of the absorbing cell. Some of these have already been studied in depth and have served as the basis for the method for measuring magnesium in serum developed in this laboratory (1). In the present paper, cation and anion interference have been studied and any problems arising from these effects have been eliminated. The effect of sample treatment and the characteristics of the burner and absorbing cell are discussed. These studies, while serving to develop a general analytical method, also explicate the basis of the observed differences in cation and anion interferences when magnesium is measured by atomic absorption spectroscopy (2, 3).

EXPERIMENTAL

Preparation of Solutions

Metal-free distilled water: water, after passage through a mixed-bed ion-exchange resin, was distilled in an all-glass still.

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Saturated oxine: 8-hydroxyquinoline (oxine) was recrystallized from a 20% alcohol-water mixture and dried under vacuum. Oxine was then slowly added to 100 ml of metal-free 1 *N* HCl until a saturated solution had formed which was then allowed to stand for 48 hr before the supernatant solution was employed.

Phosphoric acid solutions: 70% orthophosphoric acid, reagent grade, was double-distilled under vacuum. The distillate was diluted with metal-free distilled water.

Calcium, sodium, and potassium chlorides. (Johnson Matthey, Inc., London) were dissolved in metal-free distilled water.

Preparation of Standard Solutions

Spectroscopically pure magnesium (Johnson Matthey, Inc., London) was dissolved in a minimum quantity of metal-free 6 *N* hydrochloric acid and diluted with metal-free distilled water to yield a stock solution containing 10.000 μg/ml of magnesium. The stock solution was diluted further with metal-free distilled water to give solutions containing 0.1, 0.3, 0.5, 0.7, and 1.0 μg/ml of magnesium. An equal volume of saturated oxine solution was added to each of the magnesium solutions to produce the working standards.

Preparation of National Bureau of Standards Samples

National Bureau of Standards samples labeled No. 88 (dolomite) and No. 171 (magnesium-base alloy) served as certified standards of accuracy. Approximately 0.5 gm of each was weighed and dissolved in a minimum amount of metal-free 6 *N* HCl. By successive dilution with metal-free distilled water, a final magnesium concentration of 0.518 μg/ml was obtained from sample No. 88 and 0.955 μg/ml from sample No. 171. Equal volumes of these solutions were mixed with saturated oxine solution and analyzed.

For studies on interference by other ions, 0.5 μg/ml (2.06×10^{-5} *M*) magnesium solutions were prepared containing the following additions:

(A) Phosphoric acid, 1×10^{-5}, 2×10^{-5}, 1×10^{-4}, 1×10^{-3}, and 1×10^{-2} *M*.

(B) All concentrations of (A) plus 2.0 μg/ml (5×10^{-5} *M*) of calcium.

(C) All concentrations of (A) in 0.5 *N* hydrochloric acid, half-saturated with oxine.

(D) All concentrations of (A) plus 2.0 μg/ml (5×10^{-5} *M*) of calcium, 4.0 μg/ml (1×10^{-4} *M*) of potassium, and 60.0 μg/ml (2.6×10^{-3} *M*) of sodium of 0.5 *N* hydrochloric acid half-saturated with oxine.

(E) 2.0 μg/ml (5×10^{-5} *M*) of calcium, 4.0 μg/ml (1×10^{-4} *M*) o

potassium, 60.0 μg/ml ($2.6 \times 10^{-3} M$) of sodium, and $2 \times 10^{-5} M$ of phosphoric acid.

Preparation of Samples

Both human serum and cerebrospinal fluid were diluted 25 times with metal-free distilled water. The resultant solution was then diluted 1:1 with an equal volume of saturated oxine solution in 1 N HCl.

Apparatus

The apparatus employed has been described (1). Dispersion of the Zeiss monochromator (M4QIII) was 8 Å/mm at 2000 Å and 30 Å/mm at 5000 Å, and the F number was 4.5. The magnesium resonance line at 2852 Å served for analysis.

OPERATING PROCEDURE

The microammeter which displays the output of the photomultiplier tube is adjusted to zero after excluding light from the entrance slit of the monochromator. The initial light intensity, I_0, is set to 80 on the microammeter scale while atomizing a solution of metal-free distilled water and oxine (1:1). Standard solutions or samples are then aspirated and the deflection on the microammeter, I, is read. The capillary of the burner is washed between samples by aspirating metal-free distilled water before readjusting I_0 with the reference solution. Additional operating procedures have been described (1).

In the study of interference by extraneous ions, measurements were repeated at least 4 times, the relative deviations being with 3%.

RESULTS

The absorbance of an aqueous solution of magnesium, 0.5 μg/ml or $2.06 \times 10^{-5} M$, was measured in the absence and presence of increasing concentrations of phosphate (Fig. 1, curve A). Absorbance of the solution containing only magnesium is set equal to 100%. As the concentration of phosphate increases the absorbance of magnesium decreases until the ratio of Mg/H_3PO_4 is 1, but a further increase does not alter the absorbance. Absorbance of magnesium in the presence of half-saturated oxine is enhanced markedly. Moreover, under these conditions the effect of PO_4 is abolished (Fig. 1, curve B). The addition of sodium, $2.6 \times 10^{-3} M$, potassium, $1 \times 10^{-4} M$, and calcium, $5 \times 10^{-5} M$, to magnesium solutions in half-saturated oxine does not affect absorbance further, in either the presence or the absence of phosphate.

Sodium up to 300 μg/ml ($1.3 \times 10^{-2} M$), potassium up to 32 μg/ml ($8.2 \times 10^{-4} M$), calcium up to 32 μg/ml ($8 \times 10^{-4} M$), phosphoric acid

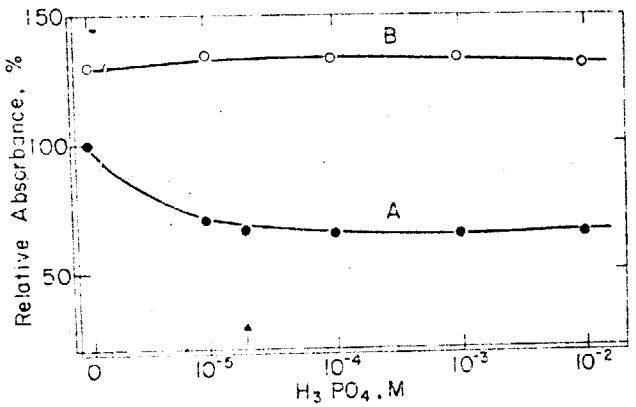

FIG. 1. Phosphate interference in measurement of magnesium by atomic absorption (curve A) and its abolition with half-saturated oxine (curve B).

up to 0.1 M, and hydrochloric acid up to 1 M, either alone or in combination do not absorb radiation at 2852 Å.

Table 1 shows the repeatability of six measurements of five different magnesium concentrations in half-saturated oxine solutions.

TABLE 1
Repeatability of Magnesium Standards Used for Atomic Absorption Working Curve

Mg concentration, μg/ml	Readings, log I_0/I						Mean ± S.D.[a]	C.V.[b] %
	1	2	3	4	5	6		
0.1	0.034	0.031	0.037	0.034	0.034	0.037	0.035 ± 0.002	6
0.3	0.104	0.100	0.107	0.100	0.104	0.104	0.103 ± 0.003	3
0.5	0.179	0.167	0.179	0.167	0.171	0.171	0.172 ± 0.006	3
0.7	0.231	0.240	0.236	0.231	0.236	0.240	0.236 ± 0.004	2
1.0	0.332	0.329	0.323	0.329	0.335	0.335	0.332 ± 0.006	2

[a] Standard deviation.
[b] Coefficient of variation.

The accuracy of the method was ascertained with N.B.S. standard samples No. 88 (dolomite) and No. 171 (magnesium-base alloy). Twenty three measurements performed over a period of three weeks demonstrated the accuracy to be 100.8% for N.B.S. standard No. 88 and 99.5% for No. 171.

The results of the repeatability and recovery of magnesium in normal human cerebrospinal fluid and serum are shown in Table 2; 0.2 meq magnesium, 0.82 meq, was added to each sample, which was then diluted 50 times and measured; in all instances the coefficient of variation is 3% or less while the recovery varies from 99.7 to 101.1%.

TABLE 2
Repeatability and Recovery of Magnesium in Normal Human Cerebrospinal Fluid and Serum

Sample	No. of analyses	Magnesium, mean ± S.D. (meq/liter)	C.V., %	Recovery, %
Cerebrospinal fluid				
A	6	2.41 ± 0.05	2	
A + 0.82 meq Mg	6	3.22 ± 0.04	1	99.7
B	6	2.18 ± 0.04	2	
B + 0.82 meq Mg	6	3.00 ± 0.04	1	100.0
Serum				
A	6	1.87 ± 0.06	3	
A + 0.82 meq Mg	6	2.72 ± 0.04	2	101.1
B	6	1.94 ± 0.04	2	
B + 0.82 meq Mg	6	2.78 ± 0.06	2	100.7

The results of triplicate analyses of both cerebrospinal fluid and serum from the same individuals are shown in Table 3. As expected, the concentration of magnesium in cerebrospinal fluid, 2.39 ± 0.24 meq/liter, is considerably higher than in serum, 1.81 ± 0.18 meq/liter.

The absorption of magnesium in an aqueous solution having the same ionic composition as serum diluted 1:50 is the same as that obtained with an identical concentration of magnesium in water alone. When

TABLE 3
Magnesium Content of Cerebrospinal Fluid and Serum from the Same Individuals

Subject[a]	Magnesium, meq/liter					
	Cerebrospinal fluid			Serum		
	1	2	3	1	2	3
1	2.48	2.37	2.41	1.95	1.89	1.89
2	2.93	2.88	2.93	2.04	1.98	2.04
3	2.21	2.14	2.21	2.00	1.95	1.95
4	2.60	2.52	2.52	1.93	1.89	1.89
5	2.49	2.32	2.28	2.14	2.04	1.98
6	2.28	2.08	2.19	1.79	1.71	1.75
7	2.60	2.49	2.49	1.79	1.71	1.71
8	2.49	2.39	2.32	1.71	1.61	1.66
9	2.19	2.04	2.08	1.61	1.61	1.53
10	2.44	2.28	2.28	1.89	1.84	1.79
11	2.71	2.54	2.49	1.61	1.52	1.53
12	2.08	2.08	2.04	1.53	1.53	1.48
Mean ± S.D.	2.39 ± 0.24			1.81 ± 0.18		
C.V., %	10.0			9.9		

[a] Subjects 1 to 5 were normal persons; subjects 6 to 12 were patients with a variety of illnesses.

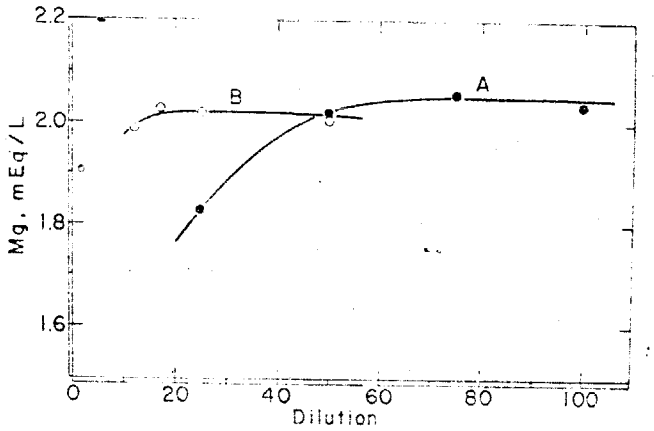

FIG. 2. Effect of degree of dilution in water on measurement of magnesium i serum by atomic absorption spectroscopy: (A) these data; (B) data of Willis (3

serum is measured in water at dilutions below 1:50 the absorption is les than that obtained when magnesium chloride is measured in the absenc of other ions or protein. However, the absorption of magnesium in serur is constant, within 3%, for all dilutions over 50-fold (Fig. 2, curve A) i accord with previous observations of Willis (Fig. 2, curve B) (3). Sinc the decreased absorption at low dilutions of serum is not due to the ioni species present in serum it may be assumed that the high protein conter

TABLE 4
Comparison of Results of Magnesium Determinations on the Same Samples after Dilution with Water or with Oxine

	Magnesium, meq/liter	
	Aqueous dilution	Oxine
Serum		
1	1.27	1.28
2	1.34	1.32
3	1.64	1.58
4	2.05	1.97
Cerebrospinal fluid		
1	0.99	2.11
2	1.05	2.75
3	1.25	2.23
4	1.29	2.12
5	1.29	2.36
6	1.30	3.76
7	1.37	2.32
8	1.55	2.24
Mean	1.26	2.49

is responsible. As shown here and previously (1), magnesium in human serum may be determined by atomic absorption spectroscopy without interference when dilution with water is the only sample treatment.

While dilution with water alone may be used to measure magnesium in serum, since the values obtained in aqueous solutions and in oxine are the same, aqueous dilutions of cerebrospinal fluid yields values that are markedly lower (mean = 1.26 meq/liter) than those measured in the presence of oxine (mean = 2.49 meq/liter) (Table 4). The ionic composition of cerebrospinal fluid which contains more magnesium, less calcium, and the same concentration of phosphate accounts for this difference (*vide infra*).

DISCUSSION

Phosphate interference in magnesium or calcium analysis by flame emission or atomic absorption spectroscopy may be attributed to the high boiling points of the alkaline earth phosphates. Since neither emission nor absorption of radiation can occur unless the atoms are in the gaseous state the emission or absorption will be reduced depending upon the relative proportion of salts having a high boiling point remaining in the solid state in the flame (4, 5).

Phosphoric acid has been reported not to interfere with magnesium absorption in the air-acetylene flame (3, 6). On the other hand, phosphoric acid has been shown to interfere with magnesium absorption (7–10) as confirmed here (Fig. 1, curve A). Since the difference in temperature of the air-acetylene flame (2250°C) and the air-hydrogen flame (2100°C) employed here is not significant (5), the variation must be due to other causes. As in emission flame spectroscopy (11), the type of burner employed is a significant variable in atomic absorption spectroscopy. Phosphate interference does not occur when atomizer burners are used since these devices permit only fine droplets to enter the flame. Total consumption burners, however, permit large droplets to enter the flame, leading to the formation of coarse particles. Such large particles have a greatly retarded vaporization rate and are thus the cause of the observed interference.

The effect of phosphate on magnesium absorption in the present system may be explained as follows: As the concentration of phosphoric acid increases (Fig. 1, curve A), the amount combined with magnesium will increase up to $2.06 \times 10^{-5} M$. At $2.06 \times 10^{-5} M$ phosphoric acid, all the magnesium combines with phosphoric acid, and further increases in phosphate concentration have no effect. In serum, since the concentration of calcium ($2.5 \times 10^{-3} M$) is 2.5-fold higher than that of either magnesium ($1 \times 10^{-3} M$) and phosphate ($1 \times 10^{-3} M$), calcium phosphate will be

formed preferentially over magnesium phosphate as the liquid droplets are dehydrated. Moreover, the solubility product of calcium phosphate also favors the precipitation of this salt. Thus when, as in serum, the concentration of calcium is greater than that of magnesium and the concentration of phosphate is relatively low, the depressing effect of phosphate on magnesium absorption is obviated. Conversely, phosphate interference would be predicted to occur when cerebrospinal fluid diluted only with water is analyzed since its concentration of calcium is lower while that of magnesium is higher. The phosphate concentration of urine is relatively high and therefore similar considerations are pertinent to the analysis of magnesium in this fluid. Indeed, phosphate ion interference must be overcome in the analysis of any biological material containing substantial amounts of phosphate or higher concentrations of magnesium relative to those of calcium, a circumstance which applies to most biological samples, e.g., tissues, urine, subcellular organelles, or microorganisms (12).

In the present instance phosphate interference has been obviated by performing the analyses in half-saturated oxine (13). The high concentration of oxine effectively competes with phosphate so that magnesium oxinate is the species which precipitates as the sample is dehydrated in the flame. Moreover, the magnitude of the logarithm of the formation constant (4.74) of magnesium oxinate which is greater by several orders of magnitude than that of magnesium monohydrogen phosphate (2.50) favors its preferential formation. As has been pointed out (14), combustion of the metal oxinate in the flame to yield metal atoms is exothermic, rendering magnesium oxinate an ideal species to be precipitated in the flame and accounting perhaps for the enhancement of magnesium absorption observed in half-saturated oxine.

These studies also demonstrate the importance of the absorbing system. In both the present and the previous work (1) measurement of serum magnesium after simple aqueous dilution results in virtually 100% recovery of added magnesium. On the other hand, recovery of greater than 100% has been consistently observed when serum is diluted only with water (3). Fishtail burners when employed as the absorbing cell (2, 3) produce a nonuniform flame. Hence, the selection of the optimal cross section of the flame from which radiation enters the optical path is critical for the measurement of magnesium absorption. The optimal cross section for the analysis of individual elements in the flame is a functio of the composition, physical properties, etc. of the sample being measured. Therefore, the optimum position of the flame for measurements magnesium absorption will be altered by a difference between standa and sample solutions. Since in the system used here the whole fla

enters the refractory tube and the total integrated absorption is measured, this problem does not exist.

SUMMARY

The effects of cations, anions, the type of burner, and absorbing system on the measurements of magnesium by atomic absorption spectroscopy have been investigated. A general method for the measurement of magnesium in biological material by atomic absorption spectroscopy has been developed which employs oxine to overcome interference due to phosphate.

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1

Simultaneous Determination of Calcium and Magnesium of Serum by a Single Chelometric Titration

Sanford H. Jackson and Florence Brown

Titration of a trichloracetic acid filtrate of serum with EDTA is carried out at pH 9.8 using an excess of Calmagite indicator. Successive photometric readings of percentage transmission, made with an EEL titrator, are plotted against the volume of EDTA added. There is very little change at first while calcium is being complexed; the reading then decreases rapidly while magnesium is being titrated, followed by a flattening of the curve at completion of the titration. Hence, two sharp "breaks" are obtained in the titration curve, the first corresponding to the amount of calcium and the second to the amount of magnesium.

IT IS EVIDENT from the variety of procedures that has been introduced for the determination of magnesium in serum that difficulties are encountered in this estimation. These difficulties have also hindered the inclusion of the determination in the repertoire of the average clinical chemistry laboratory.

The phosphate precipitation procedures, e.g., that of Simonsen *et al.* (*1*), are slow, taking at least 6 hr., but appear to give accurate results.

Titan yellow (*2, 3*) procedures are difficult to control, both because the color is colloidal and, therefore, dependent on particle size, and because the spectral absorption of the blank may constitute 75% or more of the total absorption.

Flame photometric methods, particularly that of Alcock *et al.* (*4*) may be highly accurate, but require the use of a monochromator capable of separating the 285.2 mμ line of magnesium from the 285.3 mμ line

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of sodium. Such a flame photometer is not generally available in clinical chemistry laboratories. The flame photometer of Wacker and Vallee (5), using the 371 mμ oxide line, appears to give high results since they report a mean normal value of 2.05 mEq./L., compared to the mean value of 1.67 mEq./L. found by a variety of other investigators (4).

The 8-hydroxyquinoline fluorometric method of Schacter (6) is convenient, but also appears to give a high mean normal value (2.0 mEq./L.), although a recent automated version (7) is reported to give more acceptable normal values.

Chelometric procedures, by which calcium and calcium plus magnesium are titrated separately and the difference determined, are subject to the large errors inherent in methods dependent on determining small differences. Procedures designed to limit this error by combining the separate titrations into one operation have been published. Flaschka and Ganchoff (8) titrated calcium at pH 10 in ammonia-ammonium chloride buffer using 2:2′ bis di(carboxymethyl) amino diethyl ether (BAETA) with murexide indicator, after which the magnesium in the same solution was titrated with ethylene diamine tetra acetic acid (EDTA) using Eriochrome Black. End-points were determined photometrically. The procedure was not applied to serum. Kovacs and Tarnoky (9), using "plasma-corinth B" or Eriochrome Blue SE, titrated calcium in NaOH to a visual end-point with EDTA. They then reduced the pH by the addition of hydrochloric acid and an ammonia-ammonium chloride buffer and titrated the magnesium to a second visual end-point. Calcium and magnesium values for serum agreed with accepted normals.

This report concerns a procedure whereby both calcium and magnesium are titrated with EDTA at pH 9.8 using Calmagite* indicator. The changes in color of the indicator are followed photometrically. A graph of optical transmission against volume of titrant added exhibits two "breaks"; the first corresponds to the end of the calcium titration alone, while the second completes the titration of both calcium and magnesium.

Underwood (10) listed the following conditions necessary for successful simultaneous titration of two cations: (1) the stability constants of their complexes with EDTA must be sufficiently large; (2) the constants must differ sufficiently; and (3) the spectra of the com-

*G. Frederick Smith Company, Columbus, Ohio.

plexes must permit selection of suitable wave lengths. Underwood applied this type of titration to the determination of copper and iron.

Recently, Flaschka and Sawyer (*11*) reported a procedure for the simultaneous titration of calcium and magnesium in submicrogram amounts which is similar to that to be described here. They suggested its possible application to blood serum.

Method and Materials

All glassware is soaked in 50% nitric acid overnight and rinsed well with distilled water and finally with de-ionized water.

De-ionized water is used throughout.

All reagents and standards are stored in polythene bottles.

Reagents

Calcium stock standard, 50 mEq./L. Dissolve 1.250 gm. $CaCO_3$ (Mallinckrodt primary standard) in about 50 ml. water by the careful addition of hydrochloric acid. Dilute to 500 ml.

Magnesium stock standard, 20 mEq./L. Weigh out 0.2432 gm. of metallic magnesium ribbon. Cover with water in a beaker and dissolve by the careful addition of hydrochloric acid. Dilute to 1 L.

Dilute low standards, 2.5 mEq./L. of Ca and 1 mEq./L. of Mg. Dilute 5 ml. stock Ca standard and 5 ml. stock Mg standard to 100 ml.

Dilute high standard, 7.5 mEq./L. of Ca and 3 mEq./L. of Mg. Dilute 15 ml. stock Ca standard and 15 ml. stock Mg standard to 100 ml.

EDTA solution Dissolve 5 gm. EDTA (disodium salt) in 1 L. water.

Trichloracetic acid Dissolve 10 gm. trichloracetic acid in water and dilute to 200 ml.

Calmagite Dissolve 10 mg. in 10 ml. water. Prepare fresh daily.

Ammonium borate buffer, pH 9.8 Dissolve 7.0 gm. boric acid, 6.0 gm. sodium cyanide, and 0.5 gm. of hydroxylamine HCl in water. Add 24 ml. of concentrated ammonium hydroxide. Dilute to 1000 ml. Adjust the pH to 9.8, using a glass electrode pH meter. Add a further 6.5 ml. of concentrated ammonium hydroxide. This will neutralize the trichloracetic acid and return the pH of the titration mixture to 9.8

Apparatus

1. A micrometer syringe or the equivalent.* One rotation of the micrometer screw delivers 0.01 ml.

*Agla model, Burroughs-Wellcome & Co., Tuckahoe, N. Y.

2. An EEL titrator with filter,* consisting of a photometric unit into which a magnetic stirrer is incorporated, and a separate galvanometer.

Procedure

Add 4 ml. of trichloracetic acid to 1 ml. of serum in a small tube. Cap with parafilm and mix by shaking. Let stand for 10 min. or more, and centrifuge.

Place 0.1 ml. of Calmagite in a titration cuvet. Add 0.5 ml. of clear supernatant to the cuvet, followed by 2.5 ml. of borate buffer. Introduce a magnetic stirring bar and place the cuvet in position on the titrator. Set the sensitivity so that the galvanometer reads 100% transmission. Add EDTA from the syringe, taking the galvanometer reading in %T after each 10 divisions (⅓ revolution) of the micrometer screw. The reading will remain relatively constant during this part of the titration while the calcium is being titrated. As soon as the magnesium is being titrated the %T decreases markedly after each addition of EDTA. The end-point is reached when two or three consecutive readings are the same.

The readings of %T are plotted against the titration "units," each unit being 10 divisions, or .002 ml. The best straight line is drawn through the points on the sloping part of the curve and extrapolated to intercept the 100%T line on one end and the best horizontal line through the last titration points on the other end (Fig. 1). The intercept on the 100%T line is the calcium titration while the intercept on the final titration line is the total titration of calcium plus magnesium. The titration from the calcium intercept to the final intercept is the magnesium value. These titration values are then converted to mEq./L. by reference to standard curves (Fig. 2).

To prepare the standard curves, dilute 1 ml. of each standard solution with 4 ml. trichloracetic acid. Titrate 0.5 ml. of this solution similarly to the serum titration. Plot the standard curves by joining the points with a straight line. The intercept on the abscissa is equivalent to the blank.

Experimental and Discussion

The use of the intercept of the magnesium part of the titration curve with the 100%T line (instead of with the slightly sloping initial part

*EEL model, with filter No. 607, Evans Electroselenium Limited, Halstead, Essex, England, or Gelman Instrument Company, Chelsea, Mich.

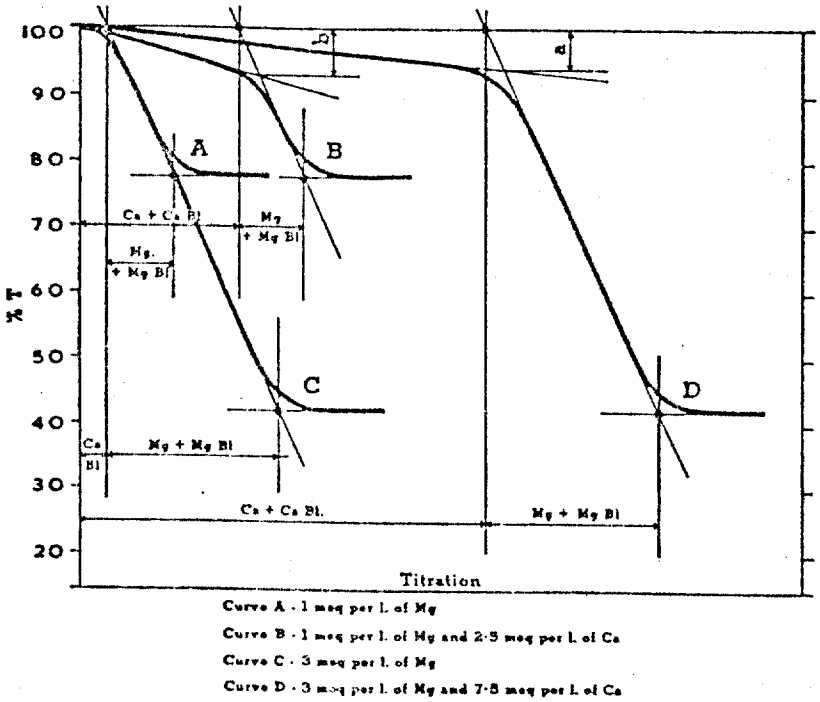

Fig. 1. Titration curves of various concentrations of calcium and magnesium.

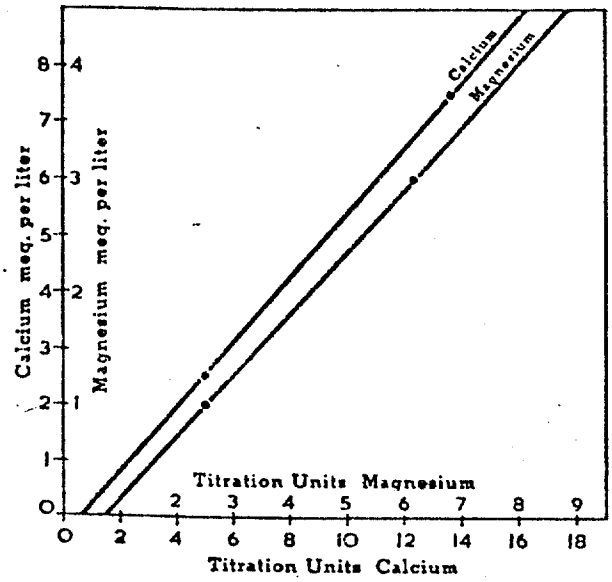

Fig. 2. Standard curves for calcium and magnesium.

of the curve) as the end-point of the calcium titration (Fig. 1) requires some explanation. There are factors other than the calcium concentration that influence this initial slope. Since the calcium and indicator have a low affinity the initial titration mixture will consist of an equilibrium mixture of calcium uncombined with indicator, free indicator, and calcium-indicator complex, as well as the firmly bonded magnesium indicator complex. The amount of free indicator, and hence the amount of calcium-indicator complex is related to the amount of magnesium. The more magnesium present, the less free indicator, and hence the less calcium-indicator complex there will be. Hence the initial slope is influenced by the magnesium as well as the calcium. This is illustrated by Curves B and D in Fig. 1. The initial drop (b) of Curve B is greater than the drop (a) of Curve D although there was three times the calcium in Titration D.

The concentration of EDTA, and hence the volume of titrant required, also affects the slope. More dilute solutions, requiring larger volumes, tend to flatten the curve by dilution of the indicator. Therefore it is advisable to use small volumes of concentrated EDTA solution.

In practice it has been observed that the use of the intercept with the initial sloping curve leads to high calcium and low magnesium results, and that the concentration of calcium will affect the magnesium determination and vice versa. However, if the intercept with the 100%T line is used, and the titrant volume is kept low, so that dilution can be neglected, the correct titration values for both calcium and magnesium are obtained.

An examination of the spectral absorbance curves (Fig. 3) of the free Calmagite indicator and its calcium and magnesium complexes reveals that the maximum spectral shift is in the 600–640 mμ region. Accordingly EEL filter No. 607, which has the transmission characteristics shown in the same figure, was selected. The transmission in the region of 700 mμ by this filter is of little consequence since the photo cell in the EEL titrator has little sensitivity at this wave length.

There is a marked effect of pH on the shape of the titration curve (Fig. 4). The optimum pH, giving the maximum spectral shift and sharpest breaks is 9.8–9.9. Departures from this pH range result in a loss of sensitivity. These curves were obtained using an ammonium borate-ammonia buffer at 0.1M concentration. Other buffers investigated were sodium borate, ammonia-ammonium chloride, monoethanolamine, and triethanolamine. Except with respect to stability there

was little to choose between them when they were used at the same pH and molar concentration. The ammonia-ammonium borate buffer was stable for at least 6 weeks. In all cases cyanide and hydroxylamine were added to complex copper and iron, the presence of which, in the

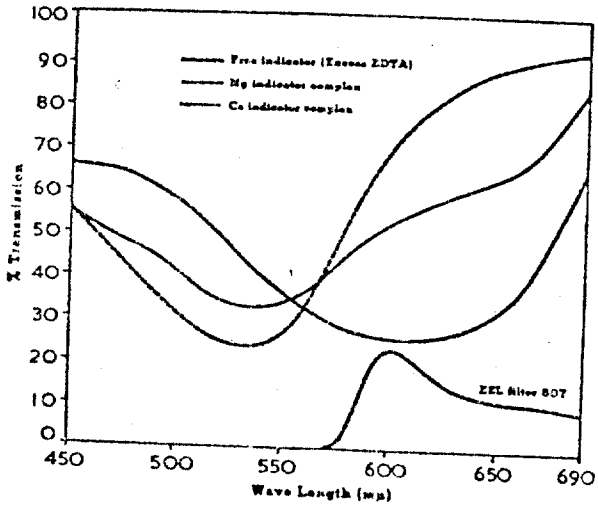

Fig. 3. Spectral absorbance curves of free indicator and in presence of excess calcium or magnesium. Also shown is spectral transmission curve of EEL filter 607.

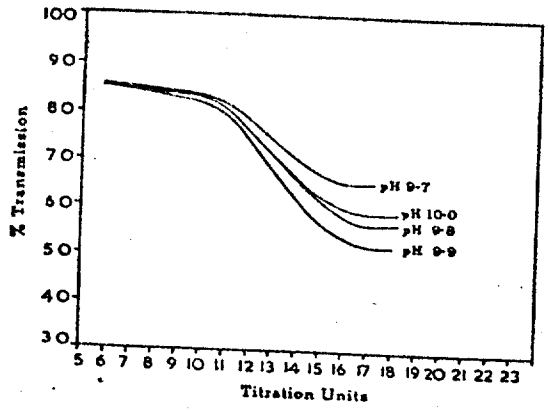

Fig. 4. Effect of pH on titration curve.

free form in minute traces, would result in a loss of definition of the breaks.

The buffer concentration has an effect on the shape of the curve, as shown in Fig. 5. The more dilute buffers lead to a greater spectral shift. The apparent increase of the calcium and magnesium titration is an artifact resulting from the increased blank values with increased reagent concentration.

The effect of indicator concentration on the titration curve is critical

magnesium present. Some excess is necessary to obtain a satisfactory calcium break. We have chosen an amount of indicator sufficient to cope with a concentration of 3 mEq./L. in the serum. If a sample should be encountered containing more than this it would be necessary to repeat the titration on a smaller aliquot.

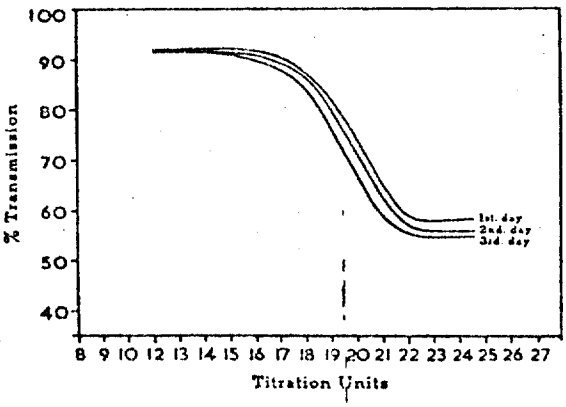

Fig. 7. Effect of age of indicator on titration curve. Ca: first day, 17.8 U.; third day, 17.4 U. Mg: first day, 4.2 U.; third day, 5.0 U. (Ca, 7.5 mEq./L.; Mg, 2.0 mEq./L.)

The effect of age of the Calmagite indicator on the titration curve is depicted in Fig. 7. There is some tendency for the calcium break to shift to the left, giving lower calcium recoveries and proportionally higher magnesium results.

Difficulties were encountered in the titration of calcium and magnesium in sera. Magnesium values were low and the slope of the curve was considerably flattened. Calcium results were satisfactory. This magnesium effect was not true of all sera, as horse serum gave a titration curve and magnesium recovery fully as satisfactory as the standards. It was suspected that the trouble arose from an effect of protein on the indicator. Figure 8 shows the effect of adding human albumin (Connaught laboratories 25% human albumin solution) to a standard titration. The flattening effect of the albumin was evident in the lowest concentration used. It was apparent that removal of the protein by precipitation with trichloracetic acid was essential. Sufficient extra ammonia was added to the buffer to neutralize the extra acid and return the pH of the titration mixture to 9.8. Standard solutions were also diluted in trichloracetic acid to maintain the same conditions of titration for both standard and tests.

A series of titrations were made in which the calcium and magnesium concentrations of the solutions were varied independently. The results are shown in Fig. 9. There was no indication that variations in

the calcium concentration affect the magnesium result or that variations in magnesium concentration affect the calcium titration.

Determinations on a series of 20 normal adults gave calcium values ranging from 4.6 to 5.4 mEq./L., with an average of 4.98 mEq./L. Magnesium values ranged from 1.40 to 1.88 mEq./L., with a mean of

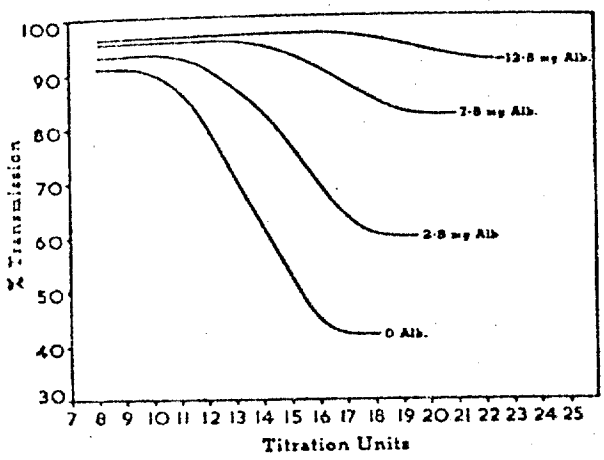

Fig. 8. Effect of human serum albumin on titration curve.

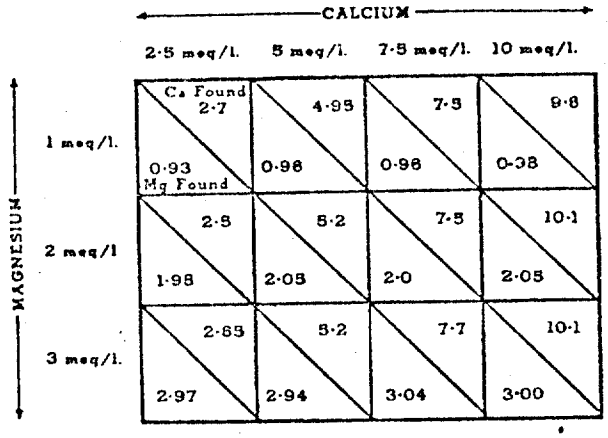

Fig. 9. Recovery of calcium in presence of magnesium, and vice versa.

1.64 mEq./L. The magnesium values agree closely with those of Alcock *et al.* (4), who reported a mean normal value of 1.66 mEq./L., with a range of 1.45 to 1.85 mEq./L.

Careful replicate determinations showed a standard error for the calcium titration of ± 0.08 mEq./L., and a standard error for the magnesium determination of ± 0.12 mEq./L., when done with duplicate titrations of the trichloracetic acid centrifugate.

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The rate of T_3 dosage increase varied, depending on age, duration and severity of hypothyroidism, and upon the response of the patient to the drug. No untoward cardiovascular manifestations developed during therapy. Determinations of serum sodium, potassium, chloride, calcium, and phosphorus and plasma and erythrocyte magnesium were carried out twice weekly. A blood urea nitrogen and CO_2 combining power were determined once weekly. Exchangeable magnesium determinations were carried out before and after a 4- to 7-week period of therapy. One hypothyroid patient was restudied with a third exchangeable magnesium determination after a 4-month period of thyroxin replacement therapy.

Constant diets and collections. The methods of preparing, storing, and processing constant diets are reported in detail elsewhere (15). Constant diets were given for a 7-day period before starting any collections. Metamucil was given daily to all patients, and distilled water enemas were utilized if necessary to close out fecal collection periods. Sweating was not observed, and no attempt was made to measure sweat electrolytes. Urines were toluolized during 24-hour collections and samples for calcium, magnesium, and phosphorus acidified to pH 3 with HCl to prevent the precipitation of insoluble salts. Stools were collected in 3-day periods in 1-gallon paint cans and diluted to a predetermined weight with demineralized water. Washed silicate stones were added, and the cans were shaken on a Red Devil paint shaker for 20 minutes. Weighed samples were promptly removed from the homogenized stool.

Analytical methods. Diet and stool samples were subjected to standard Kjeldahl digestion. Sodium and potassium were determined by emission flame photometry. Magnesium and calcium determinations were done on a Zeiss double monochromator flame photometer by the method of Macintyre (14). Erythrocytes were washed twice with and resuspended in saline, the volume of packed erythrocytes in this suspension was determined for calculation purposes, and a sample of the suspension was subjected to magnesium determinations as above. Phosphorus was determined by a modification of the method of Fiske and Subbarow (15), and nitrogen was done by the method of Peri ari (16). Urinary creatinines and blood urea nitrogen were done on a Technicon autoanalyzer. CO_2 combining powers were determined by the method of Segal (17).

^{28}Mg in the form of $MgCl_2$ was obtained,[1] and specific activity approached 20 mc per g magnesium when received. The material was neutralized with a mixture of sodium bicarbonate–sodium lactate, filtered, diluted to approximately 60 ml with sterile saline, and autoclaved. At zero time, 50 to 150 μc of ^{28}Mg was injected intravenously over a 2- to 3-minute period. Timed serum and urine samples were counted in a 2-channel well scintillation spectrometer employing thallium-activated sodium iodide crystal detectors. Automatic correction of decay of ^{28}Mg (half-life, 21.3 hours) was effected by using a stationary calibrated ^{28}Mg standard in the first channel and counting both channels to its preset count. A direct conversion to microcuries was made by multiplying the counting ratio of the unknown to the standard by the ratio of efficiency of the two detectors and the known microcurie quantity of ^{28}Mg in the standard.

The exchangeable magnesium ($^{28}Mg_E$) in milliequivalents per kilogram at 24 and 48 hours was calculated disregarding fecal loss (since it was found to be insignificant) by the following formula:

$$^{28}Mg_E = {}^{28}Mg \text{ injected} - {}^{28}Mg \text{ excreted (microcuries)} / \text{urinary specific activity (microcuries per milliequivalent)} \div \text{weight in kilograms.}$$

Cellular exchangeable magnesium pools were calculated by the method of Fankuchen, Raskin, Dimich, and Wallach (18). Methods of analysis for homogeneity of variances, standard deviations, and *t* tests are from Bailey (19).

[1] Brookhaven National Laboratory, Upton, N. Y.

TABLE I

*Plasma and erythrocyte magnesium values**

Patient	Before therapy		After therapy	
	Plasma	Erythrocyte	Plasma	Erythrocyte
	mEq/L		*mEq/L*	
Hyperthyroid				
O.B.	1.75	4.80	1.70	4.50
M.B.	1.35	5.10	1.60	5.40
V.C.	1.40	5.20	1.75	5.00
O.S.	2.00	4.60	1.75	3.80
E.L.	1.40	4.60	1.75	4.00
E.Sc.	1.42	5.60	1.60	5.00
E.W.	1.30		1.61	
B.B.	1.11			
Mean	1.51	4.88	1.68	4.61
Hypothyroid				
C.K.	2.10	4.80	2.20	5.60
E.S.	1.90	4.40	2.30	4.50
M.Sc.	1.90	4.50	2.60	4.20
M.S.	2.10	5.00	1.80	3.90
L.T.	2.00	5.50	1.75	5.80
M.Sm.	2.00	5.30	1.30	5.60
W.H.	2.30	4.80	1.90	4.30
K.S.	2.17		1.71	
Mean	2.05	4.90	1.93	4.89

* Normal values: plasma, 1.86 ± 0.14 mEq per L; and erythrocyte, 5.29 ± 0.42 mEq per L.

Results

Plasma magnesium values tended to be lowered in hyperthyroidism and elevated in hypothyroidism. PTU or T_3 therapy was associated with shifts of plasma magnesium values towards normal, with the exception of three hypothyroid patients (C.K., E.S., and M.Sc.) who had further elevation of plasma magnesium during T_3 therapy (Table I). Erythrocyte magnesium levels were significantly reduced in only one hyperthyroid patient (O.S.) before therapy and varied unpre-

TABLE II

Average daily urinary and fecal excretion in hyperthyroid patients during propylthiouracil (PTU) therapy

Patient	PTU therapy	Magnesium Excretion Urine	Magnesium Excretion Feces	Magnesium Excretion Total	Magnesium Intake	Calcium Excretion Urine	Calcium Excretion Feces	Calcium Excretion Total	Calcium Intake	Phosphorus Excretion Urine	Phosphorus Excretion Feces	Phosphorus Excretion Total	Phosphorus Intake
		mEq				*mEq*				*g*			
O.R.	Before therapy (9)*	9.57	7.31	17.18	19.80	6.08	31.17	37.25	10.00	0.938	0.351	1.312	1.531
	Day 1 to 10 (10)	8.59	8.68	17.48	18.60	4.03	27.96	31.56	36.67	0.719	0.456	1.015	1.367
	11 to 20 (12)	9.35	7.88	19.73	18.60	4.81	31.50	37.81	36.67	0.800	0.390	1.190	1.367
M.B.	Day 1 to 10 (30)	13.81	10.04	23.27	27.10	13.57	59.86	73.63	84.30	1.32	0.618	1.938	2.393
	11 to 20 (10)	11.12	7.54	18.66	26.80	9.58	40.38	50.06	93.80	1.18	0.538	1.718	2.516
	21 on (6)	10.78	9.48	20.09	27.70	5.70	31.12	36.82	93.80	1.04	0.783	1.823	2.516
V.C.	Before therapy (12)	14.70	11.75	31.05	26.90	9.93	49.12	58.05	63.20	0.927	0.910	1.837	2.170
	Day 1 to 10 (10)	13.16	11.80	24.96	26.90	7.29	59.50	66.79	63.20	0.976	1.672	2.041	2.170
	11 to 20 (10)	11.97	14.77	26.44	41.80	5.13	65.42	70.55	69.60	1.098	1.111	2.119	2.180
	21 on (11)	11.52	14.29	25.61	41.80	3.95	68.27	69.22	69.60	1.161	0.994	2.155	2.180
O.S.	Day 1 to 10 (10)	4.46	5.60	10.02	12.90	4.27	26.26	31.53	51.20	0.764	0.548	1.312	1.22
	11 to 20 (13)	4.31	8.81	13.09	19.40	5.04	56.10	61.13	56.00	0.938	0.788	1.596	1.21
V.L.	Day 1 to 10 (10)	13.01	8.31	21.15	17.60	15.09	29.59	45.58	42.40	0.938	0.439	1.377	1.06
	11 to 20 (10)	6.75	5.55	12.23	17.60	7.66	34.75	41.41	42.40	0.878	0.432	1.310	1.06
	21 on (9)	5.49	9.93	15.89	21.10	7.80	35.28	43.08	46.60	0.927	0.325	1.252	1.45

* Numbers in parentheses represent the duration in days of observations in the respective periods.

dictably after therapy (Table I). No correlation could be made between initial plasma or erythrocyte magnesium levels and the duration or severity of thyroid disease. No abnormalities or significant elevations of serum sodium, potassium, chloride, calcium, phosphorus, blood urea nitrogen, or CO_2 combining power were noted either before or during therapy.

Urinary and fecal excretion of magnesium, calcium, and phosphorus in hyperthyroid patients during PTU therapy and hypothyroid patients during T_3 therapy is shown in Tables II and III, respectively. An analysis of the data from these Tables with excretion expressed as a per cent of intake is found in Table IV. In this Table the hyperthyroid "before therapy" group includes values obtained up to and including the first 10 days of PTU therapy. Hyperthyroid patients excreted a significantly larger amount of ingested magnesium in the urine than hypothyroid patients ($p < 0.01$) before therapy, but these differences disappeared after therapy. The per cent of in-

TABLE III

Average daily urinary and fecal excretion in hypothyroid patients during triiodothyronine (T_3) therapy

Patient	Dosage of T_3	Magnesium Excretion Urine	Magnesium Excretion Feces	Magnesium Excretion Total	Magnesium Intake	Calcium Excretion Urine	Calcium Excretion Feces	Calcium Excretion Total	Calcium Intake	Phosphorus Excretion Urine	Phosphorus Excretion Feces	Phosphorus Excretion Total	Phosphorus Intake
	mg	*mEq*				*mEq*				*g*			
C.K.	Before therapy (3)*	5.33	11.53	16.53	21.30	6.67	22.50	29.17	33.00	0.464	0.200	0.664	1.28
	15 to 30 (3)	8.17	21.00	29.17	21.30	9.07	24.01	33.07	33.00	0.631	0.250	0.881	1.28
	45 to 75 (3)	12.03	18.00	30.03	21.30	11.00	29.30	40.30	33.00	0.911	0.250	1.191	1.28
	90 to 100 (25)	11.44	15.31	26.65	21.30	5.55	30.51	36.09	33.00	0.849	0.316	1.156	1.28
N.S.	Before therapy (6)	7.38	9.79	17.17	18.00	5.72	13.37	19.09	16.00	0.417	0.356	0.773	1.03
	15 to 30 (6)	4.90	9.80	14.70	18.00	5.77	11.62	17.39	16.00	0.634	0.239	0.873	1.03
	45 to 75 (13)	6.52	9.09	15.61	18.00	6.74	17.40	24.14	16.00	0.714	0.457	1.171	1.03
	90 to 100												
M.Sc.	Before therapy (2)	3.75	11.75	15.20	11.00	3.56	21.24	24.80	18.20	0.580	0.200	0.780	0.681
	15 to 30 (6)	5.03	8.57	13.60	11.00	4.25	19.55	23.80	18.20	0.688	0.135	0.823	0.681
	45 to 75 (22)	5.43	9.34	14.77	14.00	5.21	14.74	19.95	18.20	0.687	0.270	0.957	0.681
	90 to 100												
M.S.	Before therapy (3)	3.34	11.03	14.27	11.97	3.68	12.00	15.68	9.20	1.096	0.335	1.431	0.691
	15 to 30 (6)	3.99	6.48	10.47	11.97	4.49	11.72	16.21	9.20	0.817	0.216	1.033	0.691
	45 to 75 (6)	4.52	7.68	12.20	11.97	5.39	12.90	18.29	9.20	0.789	0.262	1.051	0.691
	90 to 100 (7)	4.65	9.94	14.59	11.97	6.61	23.94	30.55	9.20	0.703	0.379	1.082	0.691
L.T.	Before therapy (8)	4.08	9.34	13.42	10.40	7.16	27.96	35.12	26.50	0.511	0.396	0.907	0.750
	15 to 30 (11)	6.59	9.03	15.52	10.40	7.80	17.23	25.03	26.50	0.582	0.382	0.964	0.750
	45 to 75 (15)	7.03	7.92	14.95	10.40	7.88	30.46	38.34	26.50	0.608	0.392	1.000	0.750

* Numbers in parentheses represent the duration in days of observations in the respective periods.

TABLE IV

Analysis of excretion expressed as per cent of intake

Group	Element	Route of excretion	Per cent intake excreted: Before therapy	After therapy
Hyperthyroid	Magnesium	Urine	50.9 ± 11.5	36.5 ± 8.4 ($p < 0.02$)*
Hypothyroid			31.2 ± 8.1 ($p < 0.01$)*	46.4 ± 12.7 ($p < 0.05$)* (NS)
Hyperthyroid		Feces	43.7 ± 4.1	43.1 ± 8.1 (NS)
Hypothyroid			77.2 ± 16.1 ($p < 0.02$)	70.0 ± 12.3 (NS) ($p < 0.001$)
Hyperthyroid	Calcium	Urine	16.9 ± 9.7	10.8 ± 4.6 (NS)
Hypothyroid			28.6 ± 9.1 (NS)	40.3 ± 19.0 (NS) ($p < 0.01$)
Hyperthyroid		Feces	73.7 ± 12.7	78.9 ± 26.3 (NS)
Hypothyroid			100.8 ± 21.6 ($p < 0.05$)	126.6 ± 62.1 (NS) (NS)
Hyperthyroid	Phosphorus	Urine	57.7 ± 15.6	56.6 ± 13.6 (NS)
Hypothyroid			77.6 ± 19.8 (NS)	87.1 ± 18.0 (NS) ($p < 0.01$)
Hyperthyroid		Feces	35.4 ± 9.7	38.0 ± 15.4 (NS)
Hypothyroid			50.2 ± 14.8 (NS)	39.1 ± 14.1 (NS) (NS)

* The data have been tested for significance of differences both within and between groups, accounting for p values both horizontally and vertically within groups.

gested magnesium excreted in the urine decreased in hyperthyroids ($p < 0.02$) and increased in hypothyroids ($p < 0.05$) after therapy. The per cent of ingested magnesium appearing in the feces of hyperthyroid and hypothyroid patients did not change significantly during therapy in either group, but those with hypothyroidism excreted a significantly larger per cent of ingested magnesium in their stool both before ($p < 0.02$) and after ($p < 0.001$) therapy than did those with hyperthyroidism. Expressing the data in terms of per cent of total daily magnesium excretion appearing in stool revealed significant differences before (hyperthyroids, 48.9 ± 8.5%; hypothyroids, 71 ± 8.4%, $p < 0.002$) but not after treatment (hyperthyroids, 54.3 ± 8.6%; hypothyroids, 60.3 ± 4.9%, $p > 0.1$). A similar analysis of calcium and phosphorus data revealed statistically significant differences only in the increased fecal excretion of calcium in hypothyroid as compared to hyperthyroid patients before therapy, and in the increased urinary excretion of both calcium and phosphorus in hypothyroid as contrasted to hyperthyroid patients after therapy (Table IV).

TABLE V

Total balance data

Patient	Duration of balance	Weight change	Na	K	Mg	Ca	P	N
	days	*kg*	*mEq*				*g*	
Hypothyroid								
C.K.	34	−3.3	−304.4	−232.1	−178.24	− 87.90	+ 6.33	+ 29.10
E.S.	25	−0.8	+ 45.4	− 98.0	+ 55.90	−132.10	+ 0.63	− 60.55
M.Sc.	30	−9.1	−881.7	−416.5	− 16.90	− 85.50	− 7.21	−151.90
M.S.	22	−9.1	−562.8	−474.0	− 17.80	−264.60	− 9.18	−144.30
L.T.	34	−6.3	−441.8	−232.1	−148.50	−230.50	− 7.34	− 80.79
Hyperthyroid								
O.B.	21	−0.3	+177.1	+274.5	+ 33.32	+ 55.50	+ 5.65	+ 3.62
M.B.	26	+0.7	+421.6	+455.6	+146.10	+868.80	+17.18	+ 82.40
V.C.	43	+2.6	+146.7	+118.8	+167.19	+ 17.28	+ 5.73	+109.82
O.S.	23	+1.1	−164.6	−128.9	+ 87.30	+175.00	− 5.35	+ 4.33
F.L.	29	−0.1	+125.3	−224.7	+ 32.00	+ 10.80	− 3.92	+ 89.76

TABLE VI

*Exchangeable magnesium ($^{+}Mg_E$) at 24 hours**

Hyperthyroid					Hypothyroid				
	Before PTU therapy		After PTU therapy			Before T_3 therapy		After T_3 therapy	
Patient	24 hours	48 hours	24 hours	48 hours	Patient	24 hours	48 hours	24 hours	48 hours
	mEq/kg					*mEq/kg*			
O.B.	4.6	4.9	5.7	4.0	C.K.	3.8	4.8	4.9	
M.B.			6.2	8.3	E.S.	3.5	4.2	3.9	4.4
V.C.	4.5	7.0	5.3	6.6	M.Sc.	1.7	2.0	1.5	2.4
O.S.	5.2	6.6	5.9	6.5	M.S.	2.1	2.7	2.6	2.8
P.C.	4.1	4.3	2.8	4.8	L.T.	3.4	4.8	2.8	4.2
E.S.	4.7	7.5			M.Sm.	3.1	4.6	2.6	3.2
G.S.	3.9	6.0	3.7	3.9	W.H.	1.6		2.7	
B.B.	5.7	7.8			K.S.	3.2		3.3	
Mean	4.67	6.30	4.48	5.68	Mean	3.17	3.85	3.03	3.40

* Euthyroid = 4.3 ± 0.7 mEq per kg at 24 hours, and 6.4 ± 1.4 mEq per kg at 48 hours.

Over-all balance results are found in Table V, and representative balance graphs for hyper- and hypothyroidism during therapy are found in Figures 1 and 2. In general, during treatment the patients with hyperthyroidism developed positive balance, whereas those with hypothyroidism developed negative balance for all elements checked. Magnesium balance during treatment was invari-

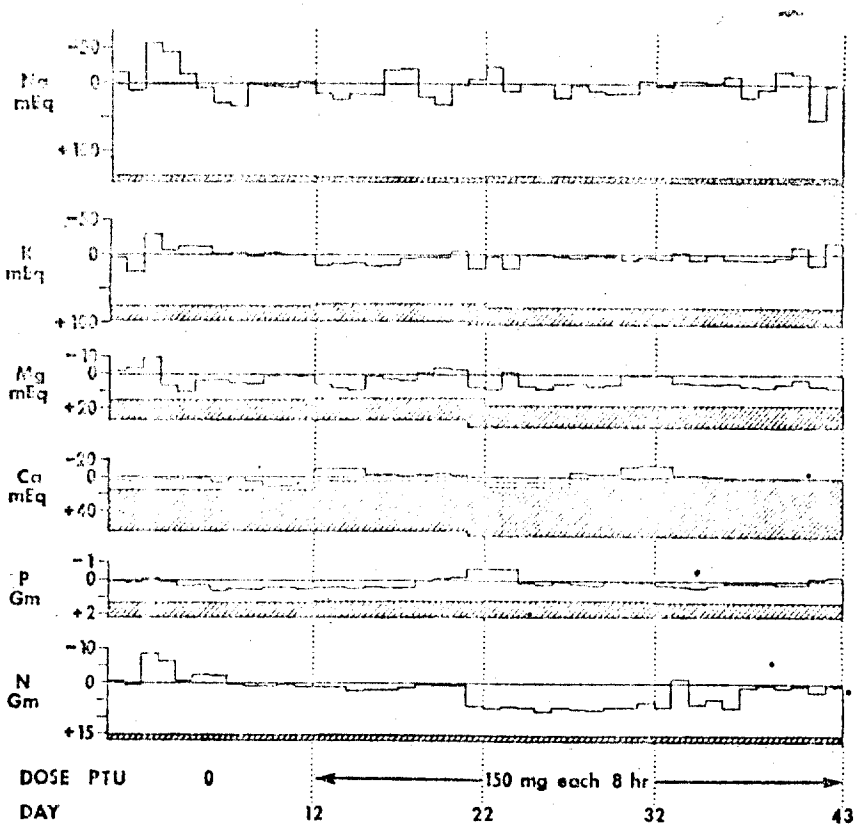

FIG. 1. REPRESENTATIVE BALANCE DATA OBTAINED BEFORE AND DURING PROPYLTHIOURACIL (PTU) THERAPY IN A HYPERTHYROID PATIENT (V.C.). Values above the zero line represent negative balance and those below, positive balance.

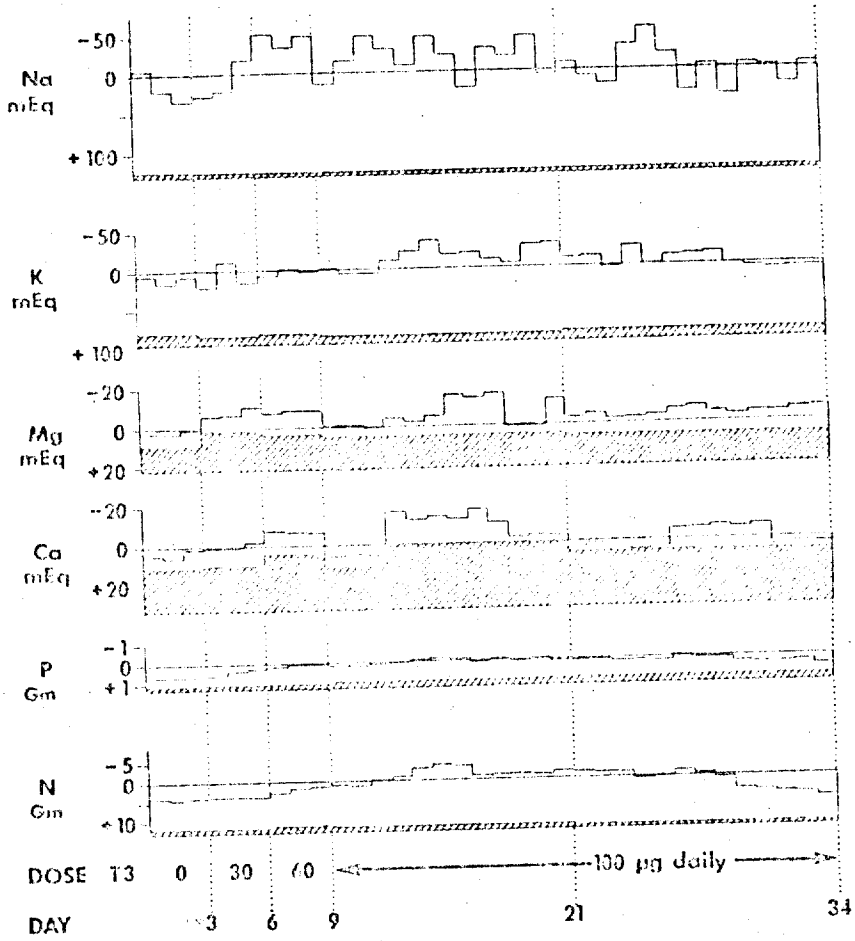

FIG. 2. REPRESENTATIVE BALANCE DATA OBTAINED BEFORE AND DURING TRIIODOTHYRONINE (T_3) THERAPY IN A HYPOTHYROID PATIENT (C.K.). Values above the zero line represent negative balance and those below, positive balance.

ably positive in hyperthyroid patients and, with one exception, negative in hypothyroid patients.

Significant differences in handling ^{28}Mg were noted between the two groups. Hyperthyroid subjects excreted significantly larger amounts of the infused ^{28}Mg in the urine at 12, 24, and 48 hours than did hypothyroid ($p < 0.01$) or euthyroid ($p < 0.05$) subjects. Hypothyroid patients excreted lesser amounts of ^{28}Mg at these time intervals than euthyroids, and the difference was significant ($p < 0.05$) at 48 hours (Figure 3). The urinary excretion of ^{28}Mg decreased after PTU therapy in hyperthyroid patients and increased after T_3 therapy in hypothyroid patients. Exchangeable magnesium values (Table VI) were normal in hyperthyroid patients before, but fluctuated somewhat after, PTU therapy (mean values normal both before and after therapy), whereas low values were obtained in six of eight hypothyroid patients before, and in seven of eight after, short-term T_3 therapy. The one hypothyroid patient restudied after a 4-month period of thyroxin replacement therapy had a normal exchangeable magnesium. No difference in cellular exchangeable magnesium was found between euthyroid and hyperthyroid patients, but hypothyroid patients were observed to have lowered values (Figure 4).

Discussion

Balance studies in hypothyroid patients given widely different doses of T_3 or thyroxin (T_4) by

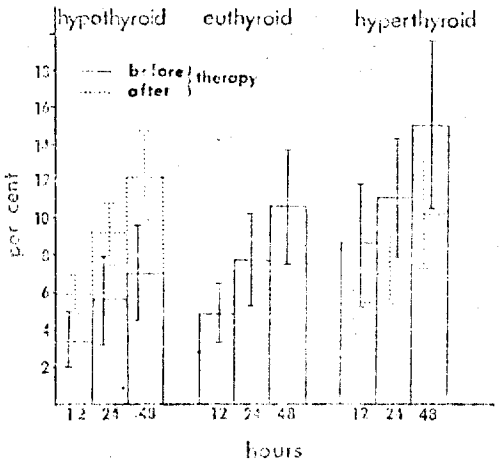

FIG. 3. MEAN (± STANDARD DEVIATION) CUMULATIVE FECAL EXCRETION VALUES OF ^{28}Mg EXPRESSED AS A PER CENT OF TOT ADMINISTERED DOSE IN HYPERTHYROID AND HYPOTHYROID PATIENTS BEFORE AND AFTER PTU OR T_3 THERAPY, RESPECTIVELY. Euthyroid values are shown for contrast.

different routes and for variable periods have been reported by several groups (20–21). Whereas no consistent changes in sodium, potassium, or calcium balances are evident in these reports, nitrogen balance tended to be negative after administration of T_4 or T_3. Munro, Kerschbaum, and Wilson (23), studying exchangeable potassium and sodium, found that PTU or surgical therapy of hyperthyroidism caused an increase in body potassium but no consistent change in sodium content, and therapy of myxedema with T_4 caused a loss of both sodium and potassium. Our results show generally positive balances for sodium, potassium, calcium, phosphorus, and nitrogen in hyperthyroid patients during PTU therapy, whereas hypothyroid patients treated with T_3 had generally negative balances for these elements. Serum sodium, potassium, chloride, calcium, phosphorus, CO_2 combining power, and blood urea nitrogen levels were normal before and showed no significant changes during therapy.

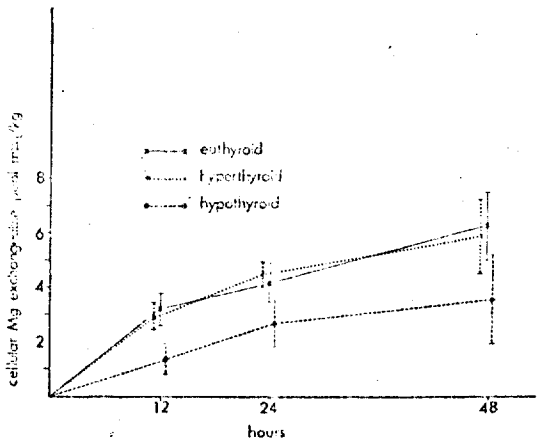

FIG. 4. CELLULAR EXCHANGEABLE ^{28}Mg VALUES IN EUTHYROID, HYPERTHYROID, AND HYPOTHYROID PATIENTS.

Tapley (24) reported a prompt increase in urinary magnesium excretion after the subcutaneous injections of 1 mg of T_3 in myxedematous subjects. A slight increase in fecal magnesium was also noted but was attributed to variation in fecal volume, since the concentration of magnesium per gram of dry stool remained relatively constant through his study. Cohen (22) included magnesium balances in two of six myxedematous subjects treated with oral T_3 in doses similar to those we employed and found essentially normal magnesium balances. Triiodothyronine therapy in our patients caused a prompt increase in urinary magnesium excretion in four of five hypothyroid patients, but no appreciable change in fecal magnesium excretion (Tables III and IV). A closer analysis of the amount of ingested magnesium excreted in the feces demonstrated a statistically significantly increased excretion in hypothyroid as compared to hyperthyroid patients both before and after therapy (Table IV). Whereas there was a decrease in fecal excretion of magnesium in hypothyroid patients after T_3 therapy, the decrease was not of sufficient magnitude to be statistically different from pretreatment levels. Fecal volume and number of stools per day were greater in hyperthyroid than in hypothyroid patients; thus the increased fecal excretion of magnesium in the latter group cannot be attributed to volume alone. An analysis of fecal calcium excretion showed changes similar to those seen with magnesium, whereas fecal phosphorus excretion was unchanged by therapy and essentially identical in both groups. An analysis of fecal magnesium excretion expressed as a per cent of total daily magnesium excretion showed significant differences between the groups before but not after therapy, suggesting that if our observations had been continued until magnesium balance was achieved the fecal magnesium in hypothyroid patients might have decreased.

Tibbetts and Aub (26) reported positive magnesium balance in two patients with exophthalmic goiter with urine excretions ranging from 1% to 48% and fecal excretion from 27 to 40% of the magnesium intake. Observations before therapy were obtained in only two of our hyperthyroid patients, and both had positive magnesium balances. The negative balances and the increased urine excretion of calcium and phosphorus found by Tibbetts and Aub were not seen in our patients and none had elevated serum calciums. The positive calcium and phosphorus balance (Table V) during therapy in our hyperthyroid patients is compatible with a negative balance before therapy; the same can be said for sodium, potassium, and nitrogen in most of the hyperthyroid patients studied. Our observations before starting PTU therapy are limited, and the possibility that the balance results might be due to a direct effect of PTU seems unlikely but cannot be excluded.

Consideration must be given to the influence of the magnesium intake and the character of the diet on magnesium balance. Seelig (27) in an exhaustive review of previously published magnesium balance data, concluded that the reported magnesium requirement of 18 to 25 mEq per day (220 to 300 mg) is low and that with intakes below 0.5 mEq per kg per day (6 mg per kg), negative magnesium balance is likely to develop, particularly in men. High intakes of protein, calcium, vitamin D, or alcohol were found to impede retention or to increase the requirement of magnesium, especially in those on low magnesium intakes. That the absorption of magnesium in normal subjects is more avid from diets of low magnesium content is suggested by the ^{28}Mg studies of Graham, Caesar, and Burgen (28). In the patients reported herein, as would be expected, hypothyroid patients selected diets lower in calories as well as protein, calcium, and magnesium than did hyperthyroid patients. One might, therefore, expect to find less of the ingested magnesium appearing in the feces in our hypothyroid than our hyperthyroid patients—an expectation just opposite to our findings. Furthermore, a comparison of our data to composite balance data and graphs of Seelig (27) indicates that our results cannot be explained on the basis of intake alone. The magnitude of negative balance in our hypothyroid patients during therapy exceeds any recorded for normal subjects on similar intakes of magnesium and calcium, and our hyperthyroid patients had strikingly positive balances in contrast to those reported in normal subjects on similar magnesium and calcium intakes. Our experience in normal adults indicates that the intake of magnesium in select diets frequently reaches levels of 0.5 mEq per kg per day and that negative balance is regularly observed with intakes from 0.25 to 0.35 mEq per kg. Fecal excretion of ingested magnesium on intakes in these ranges does not often exceed 60% and is usually lower. Whereas no data exist concerning magnesium losses in sweat in controlled temperature and humidity such as are found in a metabolic study center, Seelig (27) has estimated losses to be 1.25 mEq per day (15 mg per day) under temperate conditions. Although this figure would appear to be maximal to us, accepting such estimates of loss of magnesium in sweat would fail to alter significantly the results of magnesium balance reported.

In 1956, Zamcheck, Bernstein, Iacrisio, and Dillman (29) reported "departures from the normal pattern" of magnesium kinetics in myxedema but gave no details. Aviolí, Lynch, and Berman (30), reporting digital computer compartmental analyses of ^{28}Mg kinetics in thyroid disease, concluded that a selective decrease in exchangeable magnesium existed in "extracellular fluid," "muscle," and "bone" in hypothyroidism. In hyperthyroidism, exchangeable magnesium was selectively decreased in muscle with a two- to threefold increment in flux rates. Propylthiouracil therapy in one hyperthyroid patient was associated with an increase in muscle exchange and a decrease in flux rates. Dimich, Rizek, and Wallach (31) more recently analyzed ^{28}Mg plasma falloff curves and concluded that thyroid hormone stimulates magnesium exchange by altering cellular transport mechanisms.

Total exchangeable magnesiums were normal before and after therapy in our hyperthyroid patients with the exception of minimal elevation in one (M.B.) and reduction in another (F.L.) after PTU therapy (Table VI). The total exchangeable magnesium in hypothyroid patients was low in six of eight before therapy and in seven of eight after therapy. Patient M.S. was restudied after a 4-month period of replacement thyroxin

therapy, and her exchangeable magnesium was then found to be normal (3.87 mEq per kg at 24 hours, 6.39 mEq per kg at 48 hours). We assumed that the explanation for the low post-T_3 therapy magnesium exchanges lies in the short periods of therapy before restudy. Calculations of the cellular magnesium exchangeable pools revealed low values in hypothyroid patients. In contrast to the reports of elevated cellular magnesium exchangeable pools in hyperthyroidism of Sjaastad and co-workers (18), we found no difference between euthyroid and hyperthyroid individuals (Figure 4).* Allawa (32), studying magnesium metabolism in rabbits, found a decrease in exchangeable body content of magnesium and a decrease in the relative ^{28}Mg activity in vertebrae after prolonged PTU therapy. Injections of thyroxin in animals caused no measurable changes in magnesium exchanges but did result in an increase in the relative ^{28}Mg activity in bone, skin, appendix, and heart. In normal humans little contribution to the exchangeable magnesium is made by bone (30). It is possible, however, that in the face of hyperthyroidism the retention of bone to measured exchanges may be greater than suspected on the basis of ^{28}Mg exchange total pools utilized to date. The magnitude of positive magnesium and calcium balance was observed in hyperthyroidism during therapy would be in keeping with a significant contribution of bone in the over-all balance. If an increase in rate of flux of magnesium into muscle does occur (cf. ref. 33), it is conceivable that the larger dietary intakes of magnesium in hyperthyroidism might function to maintain normal, albeit rapidly exchanging, cellular magnesium levels. The studies of Johnson, Posey, Patrick, and Caputto (33) of ^{32}P incorporation in the muscle of thyrotoxic rats suggest a faster than normal penetration of inorganic phosphorus but an impeded incorporation into organic phosphorus intracellularly. Magnesium transport might similarly be heightened but its intracellular utilization impeded in hyperthyroidism.

It is possible that the mucopolysaccharide of myxedema is capable of polyionic binding and that the negative electrolyte balances seen during therapy are due to the thyroid hormone-induced decreased binding as a result of dissolution of this mucopolysaccharide. Such a hypothesis leaves questions unanswered, at least in relation to magnesium metabolism. The increased excretion of ingested magnesium in the feces, the decreased excretion of magnesium in the urine, and the lowered total and cellular exchangeable magnesium in the face of elevated levels of plasma magnesium strongly suggest that transport difficulties of magnesium across cell membranes occur in hypothyroidism. Known examples of thyroxin influence on transport mechanisms include the thyroxin-induced increase in transport of glucose across the intestine of hamsters (31), the thyroxin-induced increase in water transport across the isolated toad bladder (35), and the thyroxin-induced accelerated cellular penetration of ^{32}P in rats (33).

Summary

Eight hyperthyroid and eight hypothyroid patients were studied by determinations of serum and erythrocyte magnesium, exchangeable magnesium, and total balance studies. Hyperthyroid patients were found to have decreased plasma magnesium, increased urinary excretion of ^{28}Mg and ^{28}Mg, and normal total and cellular exchangeable magnesium before therapy. Hypothyroid patients had elevated plasma magnesium, decreased urinary ^{28}Mg and ^{28}Mg excretion, increased fecal magnesium excretion, and strikingly reduced total and cellular exchangeable magnesium before therapy. Erythrocyte magnesium values were normal in both groups. After propylthiouracil or triiodothyronine therapy, plasma magnesium levels shifted towards normal, and urinary excretion of ^{28}Mg and ^{28}Mg was reduced in hyperthyroid and increased in hypothyroid patients. Total and cellular exchangeable magnesium values remained normal in hyperthyroid and low in hypothyroid patients after short-term therapy. The exchangeable magnesium was found to be normal in one hypothyroid patient restudied after a 4-month period of thyroxin replacement therapy. Over-all balances of sodium, potassium, calcium, phosphorus, and nitrogen during therapy were generally positive in hyperthyroid and negative in hypothyroid patients. Magnesium balance was invariably positive in hyperthyroidism and, with one exception, negative in hypothyroidism. The data suggest that a defect in magnesium transport occurs in thyroid hormone deficiency states.

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Magnesium Metabolism in Hyperthyroidism and Hypothyroidism *

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In 1939, Hunter (1) reported clinical improvement in patients with thyrotoxicosis after parenteral administration of magnesium. Wisneski (2) subsequently was unable to demonstrate any change in the peripheral metabolism of thyroid hormone in hyperthyroid patients given magnesium sulfate injections, whereas Naguib (3) reported a decrease in size of both tonic and clonic goiters and clinical improvement in three previously resistant patients given daily injections of magnesium chloride. Lipsky (4) demonstrated that parenteral infusion of triiodothyronine promptly produced negative magnesium balance in two hyperthyroid patients and called attention to the similarity between the symptoms of thyrotoxicosis and magnesium deficiency, as well as between hypothyroidism and magnesium excess. That the serum magnesium is elevated in hypothyroidism and depressed in hyperthyroidism has long been known (5), but has recently been re-emphasized and associated erythrocyte magnesium alterations reported (6).

Lempsky and Astwood (9) demonstrated increased production of thyroid hormone in animals maintained in the cold. Hegsted, Vitale, and Nakamura (10) reported that the magnesium requirement to maintain maximal growth rates in rats kept at cold temperatures were four times those of animals kept at normal temperatures. Vitale, Hegsted, Nakamura, and Connors (5) later reported that the growth inhibition caused by the addition of thyroxin to the diet of young rats could be partially overcome by extra supplements of magnesium. Magnesium inhibits the action of thyroxin on uncoupling of oxidative phosphorylation *in vitro*. Conflicting reports exist as to the influence of magnesium deficiency on oxidative phosphorylation (11, 12).

The present investigation was carried out to assess further the relationship between the functional status of the thyroid gland and magnesium metabolism. Hyperthyroid and hypothyroid patients were studied by determinations of serum and erythrocyte magnesiums, exchangeable magnesiums, and by complete balance studies during therapy with propylthiouracil and triiodothyronine, respectively.

Methods

Clinical materials. Eight hyperthyroid and eight hypothyroid patients were studied in our clinical center for metabolic studies. Clinical findings were charted, and the diagnoses were confirmed by determinations of protein-bound iodines, 24-hour ^{131}I uptakes, basal metabolic rates, and, in selected hypothyroid patients, repeat ^{131}I uptakes after the intramuscular administration of thyroid-stimulating hormone. None of the patients had congestive heart failure or were receiving diuretic agents. Two hyperthyroid patients were taking digitalis for control of arrhythmias at the time of referral, and this drug was given in maintenance doses throughout the studies in these two patients. Our clinical center is centrally heated and air conditioned and has a relatively constant temperature of 24° C and relative humidity of 30%. Activity was minimal, restricted to the clinical center, and essentially identical from day to day throughout the course of the studies. Balance studies of 21 to 43 days were carried out in five hyperthyroid patients during propylthiouracil (PTU) therapy and in five hypothyroid patients during triiodothyronine (T_3) therapy. PTU was given each 8 hours in doses of 150 to 200 mg.

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A preliminary report has appeared (Proceedings of the Central Society for Clinical Research 1965, 38, 39).

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The Automated Fluorometric Determination of Serum Magnesium

II. Procedure Using 8-Hydroxyquinoline-5-Sulfonic Acid

Bernard Klein and Morris Oklander

Two automated flow systems are presented for the fluorometric determination of serum magnesium. Each procedure is based on measurement of the fluorescence of magnesium 8-hydroxyquinoline-5-sulfonate. One method treats a serum dialysate; the other reacts serum directly with the aqueous reagent. Calcium interference is eliminated by sequestration.

A PREVIOUS COMMUNICATION from this laboratory (*1*) reported modifications and improvements of the Hill automated procedure (*2*) for the fluorometric determination of serum magnesium. A new automated procedure, now presented, is based on measurement of the characteristic fluorescence of magnesium 8-hydroxyquinoline-5-sulfonate solutions (*3, 4*).

Two flow systems have been developed. The first also incorporates dialysis prior to reaction with the reagent. The second, without dialysis, uses only 0.1 ml. of serum in direct reaction with the fluorogenic reagent. Each procedure offers the advantage of operation in wholly aqueous solution with a sensitivity equal to or greater than that obtained with the earlier methods.

Experimental Technic

Reagents

Tris buffer, 0.1 M, pH 8.0 12.1 gm. of tris(hydroxymethyl)aminomethane in a liter of solution, is adjusted to pH 8.0 with 5 N HCl.

8-Hydroxyquinoline-5-sulfonic acid

1. Stock reagent, 0.05 M 1.13 gm. of 8-hydroxyquinoline-5-sulfonic acid (Distillation Products Industries no. 4666) is dissolved in

From the Automation Research Laboratory, Veterans Administration Hospital, Bronx, N. Y. 10468.

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cence response should be between 60 and 80 arbitrary units. If less than 60 or above 80, the slits are replaced to bring the response within this range. The base line is readjusted to the 1% line if necessary. The analyst is referred to the fluorometer operating instruction manual for details.

Standard magnesium solutions and specimens are then aspirated at the rate of 40 samples per hour (2:1 wash ratio), and the recordings of their fluorescent response are obtained (see *Discussion*).

Calibration Curve

Standard magnesium solutions containing 0.5–4.0 mg. Mg^{++}/100 ml. (0.41–3.28 mEq./L.) are analyzed and the fluorescence response, in arbitrary units, is plotted against concentration. Expression as milliequivalents per liter is readily obtained by multiplying milligrams of Mg^{++} per 100 ml. by 0.82. In the discussion which follows, wherein the elements contributing to the development of the present method are examined, Mg^{++} concentrations are expressed as milligrams per 100 ml.

Results

Figure 3 shows a typical calibration curve and the strip chart recording from which it was constructed. It is evident that the fluorescence response (in arbitrary units) is linear to 4.0 mg. Mg^{++}/100 ml. Also shown are the continuous sampling ("steady state") recordings. The fluorescence responses obtained at 40 specimens per hour (2:1 wash ratio) are 98%, 98%, 99%, and 98% respectively. It will also be noted that in this series there is no interaction when the 4.0 mg. Mg^{++} stand-

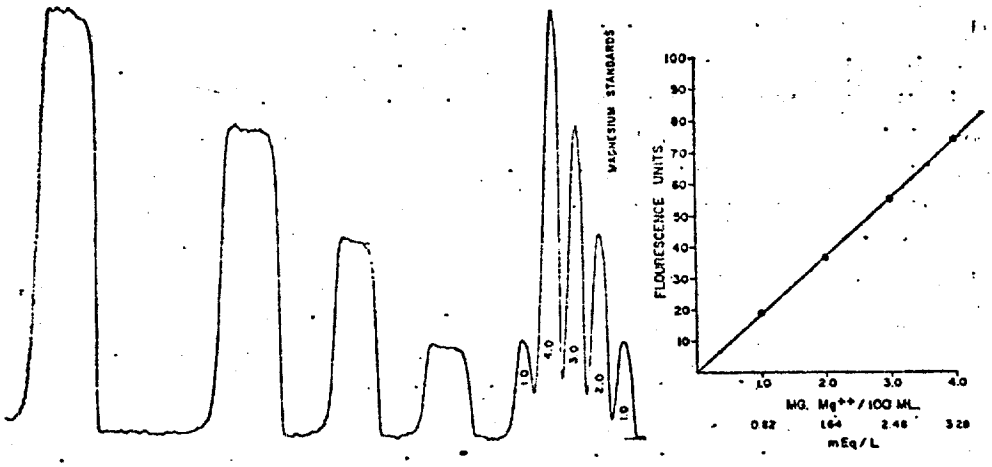

Fig. 3. At right is calibration curve, fluorometric serum magnesium determination with dialysis. At left are continuous sampling recordings.

ard is followed by the 1.0 mg. Mg^{++} standard. In other experiments during the development of this procedure, a maximum interaction of 3% was observed, under similar conditions.

Discussion

The present procedure was developed as a preliminary to a contemplated examination of the role of Mg^{++} in neurologic, renal, and hepatic disorders. Although the previously described modification (*1*) of the Hill automated fluorometric procedure was useful in early pilot studies, the instability of the 8-hydroxyquinoline reagent in ethanolic solution detracted from its utility. The more stable aqueous 8-hydroxyquinoline-5-sulfonate reagent used in the present procedure also offered advantages in that it (1) eliminated the need for Solvaflex tubing; (2) simplified the manifold and flow diagram characteristics; (3) could be used with either flow system; and (4) was more sensitive—e.g., in the direct procedure, less serum sample was required to give the same fluorometric response (*3*).

In the earliest experiments with the aqueous reagent (Fig. 1), it was soon observed that the presence of Ca^{++} introduced an appreciable increase in total fluorescence, although Schachter (*3*) had reported that at pH 7.0, Ca^{++} solutions produced negligible fluorescence with the aqueous reagent. This effect is shown in Fig. 4, on the right. Following the recordings of the fluorescence of Mg^{++} solutions (first 5 peaks), the fluorescence response is demonstrated of 2.0 mg. Mg^{++}/100 ml. samples to which Ca^{++} equivalent to 5.0, 10.0, 15.0, and 20.0 mg./100 ml. had been added. The increase in fluorescence is 7.5, 11.2, 11.8, and 12.2% respectively. Shown below is the fluorescence response of the control solution containing only diluent buffer and reagent, demonstrating that the additional fluorescence is due to the Ca 8-hydroxyquinoline fluorophore. The last recording is that given by a solution containing [illegible] ml. Ca^{++}. Its fluorescence is equivalent to 1.03 mg. Mg^{++}/100 ml. This seeming discrepancy between Schachter's data and those obtained in this laboratory is in part the result of instrumental differences. Schachter measured the fluorescence in a spectrophotofluorometer with the peak response at 510 mμ. In the present procedure, the broader spectral transmission resulting from the use of a filter photofluorometer allowed fluorescent nonmagnesium contributions to be recorded. This problem also existed in the Hill procedure and was solved by incorporation of potassium oxalate (2 mg./ml.) into the diluent buffer prior to dialysis. Incorporation of potassium oxalate into the diluent of the present system (Fig. 1) did not produce the desired

effect. The reaction of the dialysate with the aqueous reagent was sluggish, with reduced (19.5%) fluorescent response, even with increased reagent concentration. Furthermore, at 2.0 mg. Mg^{++}/100 ml. for example, sample interaction increased to 11% and an increase in nonreagent blank fluorescence also resulted. When identical serum specimens were analyzed by the potassium oxalate-modified automated procedure and the Schachter manual procedure (*3*), the former, in some instances, gave Mg^{++} values as much as 20% higher than the latter, signifying the continued presence of fluorescence-producing interference. Increasing the potassium oxalate above 2 mg./ml. in buffered diluent caused Mg^{++} binding. This also occurred upon the addition of oxalate to the recipient stream. The use of potassium oxalate was discontinued.

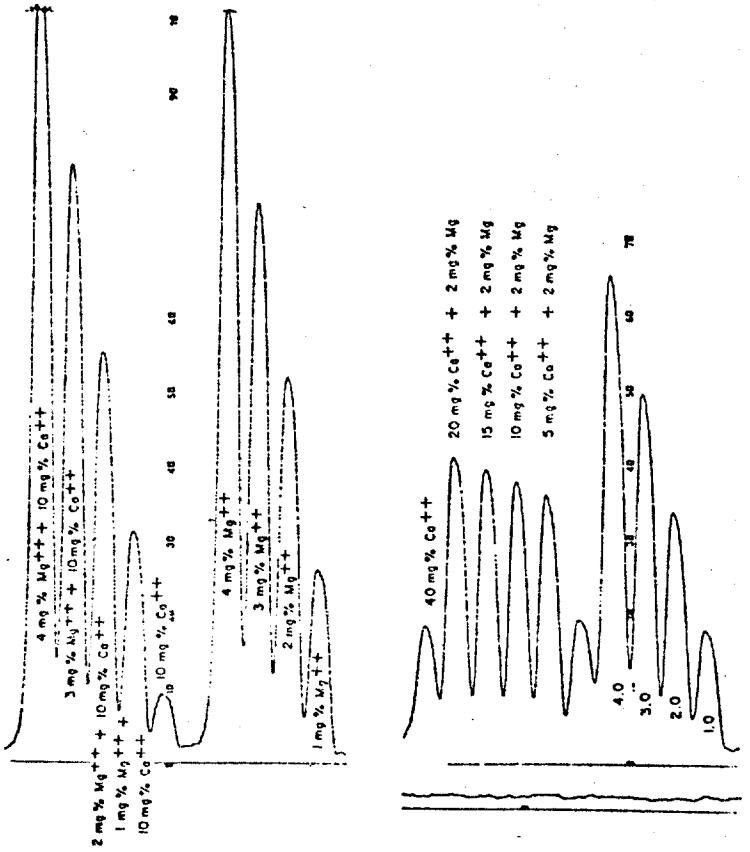

Fig. 4. Fluorometric serum magnesium determination, showing effect of Ca^{++} on Mg 8-hydroxyquinoline sulfonate fluorescence. Recordings at left were obtained by increasing instrument sensitivity to illustrate effect of Ca^{++} fluorescence enhancement. Serum control recordings are below recordings at right. 1° = 7-60; 2° = 2A-12; Slit 3; magnification, × 10; RT, pH 6.0; Sample 0.23.

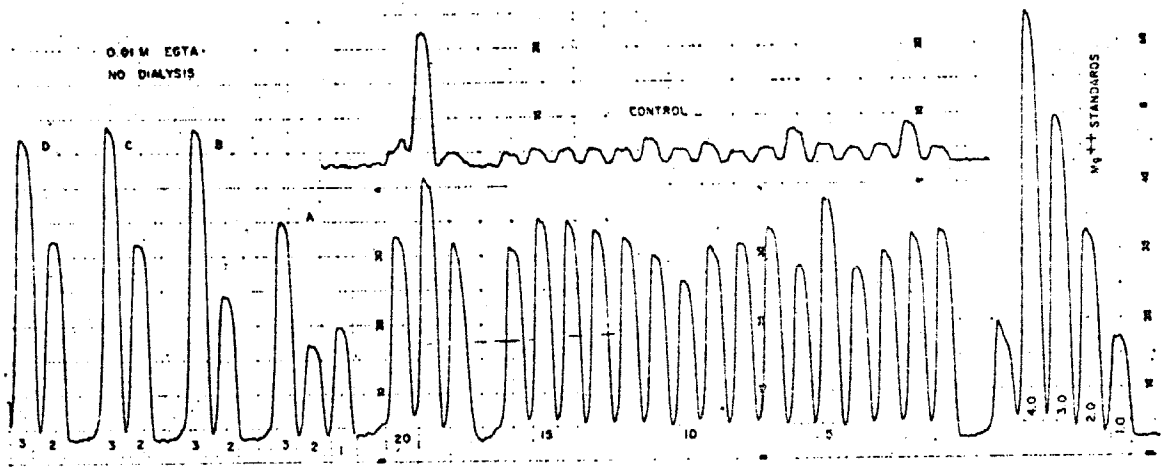

Fig. 5. Fluorometric serum magnesium determination. For recordings of analyses by direct reaction, 10^{-2} M EGTA was used to sequester Ca^{++}; reagent concentration was 7.5×10^{-3} M. Serum control recordings are shown above recordings of specimens.

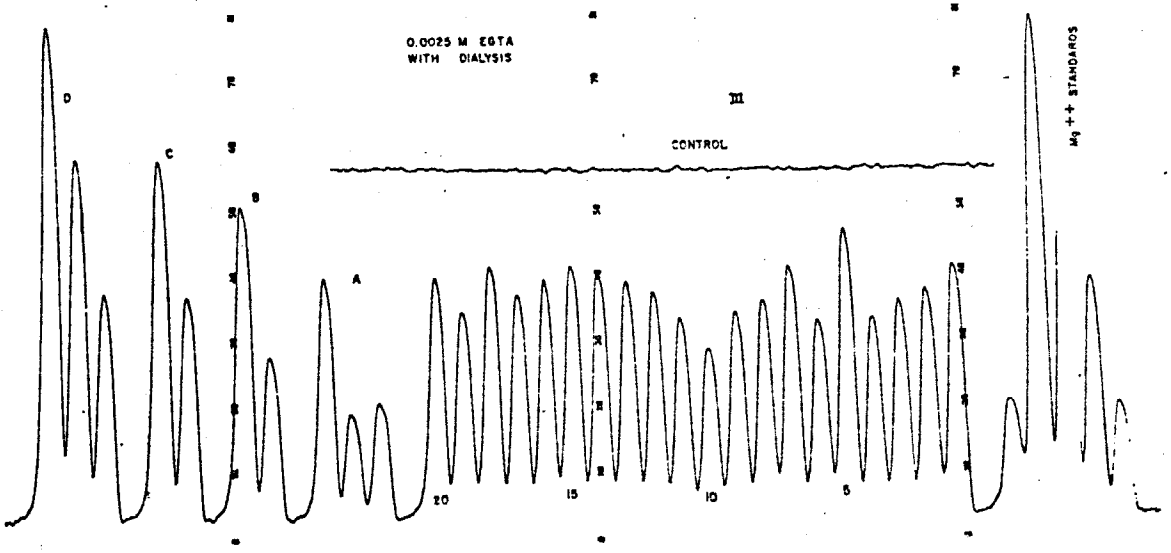

Fig. 6. Fluorometric serum magnesium determination. For recordings of analyses by dialysis procedure, 2.0×10^{-3} M EGTA was used to sequester Ca^{++}; reagent concentration was 2.5×10^{-3} M. Serum control recordings are shown above recordings of specimens.

Very recently, Lamkin and Williams (*6*) reported that EGTA effectively sequestered Ca^{++} in the presence of Mg^{++}. This reagent was substituted as the complexing agent in the present procedure and proved effective. The optimum EGTA concentration for the sequestration of up to 25 mg./100 ml. Ca^{++} in the presence of 2 mg./100 ml. Mg^{++} was determined. In 2×10^{-3} M EGTA (pH 8.0), Ca^{++} at concentrations of 6.3, 12.5, 18.8, and 25 mg./100 ml. produced fluorescence intensities of 0.5, 0.5, 1.0, and 1.0 units respectively, above the base line. Above 3×10^{-3} M EGTA, Mg^{++} is also sequestered under the present experimental conditions.

One other modification introduced at this time was the substitution of the No. 2A-12 filter for the No. 58 as the secondary filter. The former, a combination of Wratten 2A and Wratten 12, is a sharp-cut filter passing wave lengths longer than 510 mμ and yielded a 35% increase in fluorescent response.

The dialysis is conducted at room temperature. It was observed that the warm reaction stream emerging from the dialyzer module at 37° produced a lower fluorescent response. Reduction of fluorescence with temperature elevation is recognized (*7*). Variations in dialysis rate will occur with gross changes in dialyzer bath temperature, but over a 2-week period, the temperature in the unregulated water bath did not vary by more than 3° in this partly air-cooled laboratory.

The possibility arose that serum magnesium could be determined by direct treatment of serum diluted with Tris buffer containing EGTA, with the aqueous reagent, since Schachter had not deproteinized the serum in his procedure (*3*). The system diagramed in Fig. 2 was assembled. To sequester the calcium of whole serum, 0.01 M EGTA was required. The hydroxyquinoline sulfonate reagent concentration was also increased to 7.5×10^{-3} M. The fluorescence of standard magnesium solutions analyzed by this procedure was also linear with concentration (Fig. 5).

To determine what error was introduced by the elimination of dialysis, 20 serum specimens were analyzed by both procedures. The recordings in each analysis are shown in Fig. 5 and 6. Also shown in each series are the recordings of the fluorescence produced by the individual serums similarly analyzed with EGTA to sequester Ca^{++} in the diluent stream but only buffer pumped in the reagent line. In the nondialyzed series (Fig. 5), the blank fluorescence usually did not exceed 3–4 units, equivalent to 0.28 mg. Mg^{++}/100 ml. In the dialyzed series, this was about 0.5–1.0 unit (Fig. 6). In one instance, Specimen 19, the blank serum fluorescence was 19.5 units. Similar fluorescence was not observed in the dialyzed serum analysis. This would indicate the presence

of a nondialyzable fluorescing serum constituent of undetermined nature. It can be concluded that usually a 4-unit correction can be applied to the fluorescence recorded in the direct procedure (Fig. 2) to obtain results in agreement with the Mg^{++} concentrations obtained in the dialysis procedure (Fig. 1). The serum Mg^{++} concentrations de-

Table 1. Magnesium Analyses by the Automated 8-Hydroxyquinoline-5-sulfonate Procedure with Dialysis and by Direct Reaction

	Mg^{++} (mEq./L.)		
Specimen	*Dialysis*	*Direct*	*Difference*
Serum pool	1.72	1.56	0.16
1	1.56	1.31	0.25
2	1.47	1.39	0.08
3	1.39	1.23	0.16
4	1.97	1.72	0.35
5	1.39	1.23	0.16
Serum pool	1.72	1.56	0.16
6	1.47	1.47	0.0
7	1.43	1.39	0.04
8	1.23	1.31	0.08
9	1.39	1.31	0.08
10	1.60	1.48	0.12
11	1.68	1.56	0.12
Serum pool	1.68	1.64	0.04
12	1.80	1.40	0.40
13	1.68	1.48	0.20
14	1.60	1.60	0.0
15	1.72	1.48	0.24
16	1.39	0.99	0.40
Serum pool	1.69	1.40	0.29
Ave. difference			0.17

Table 2. Recovery Analyses, Automated 8-Hydroxyquinoline-5-sulfonate Procedure, by Dialysis and by the Direct Reaction

	Dialysis method				*Direct reaction method*			
Specimen	*Mg^{++}*	*Mg^{++} added*	*Total Mg^{++}*	*Recovery (%)*	*Mg^{++}*	*Mg^{++} added*	*Total Mg^{++}*	*Recovery (%)*
Serum pool A	0.82	0.82	1.64	100	0.90	0.82	1.72	100
Serum pool B	0.82	1.23	2.05	100	0.90	1.23	2.21	104
Serum pool C	0.82	1.64	2.46	100	0.90	1.64	2.46	96.7
Mg solution with Ca^{++} (10 mg./100 ml.)		1.64	1.56	95	—	—	1.60	97.5
Mg solution with Ca^{++} (10 mg./100 ml.)		2.46	2.46	100	—	—	2.38	96.7

All amounts are given in milliequivalents per liter.

termined by both procedures are given in Table 1. Good agreement was observed. The direct analysis is offered as a procedure which will permit reliable serum magnesium analysis, provided the limitation cited above is recognized and the average 4-unit correction is applied. In the few instances where unusually elevated serum Mg^{++} concentrations are observed, the actual blank serum correction can be easily determined as described above. In contrast, the preparation and maintenance of a dialysis module, with its attendant problems, must be considered.

Following the series of serum analyses in Fig. 5 and 6 are the recordings obtained in a series of recovery analyses in which known amounts of Mg^{++} were added to a serum pool. This is followed by a group of analyses of magnesium standard solutions each containing 10 mg. Ca^{++}/100 ml. The results of these experiments are summarized in Table 2.

During the developmental phase of this procedure, specimens were aspirated at the rate of 40 samples per hour (2:1). More recent experiments demonstrated that sampling at a rate of 60 specimens per hour (2:1) gave fluorescent responses 95–97% of those obtained at the 40/hr. rate. The results obtained at both sampling rates were identical.

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1.

SCIENTIFIC NOTES

Automated Atomic Absorption Spectrophotometry

II. The Determination of Serum Magnesium

Bernard Klein, James H. Kaufman, and Morris Oklander*

The flow system developed for the determination of serum calcium by automated atomic absorption spectrophotometry (AAS) was adapted to the determination of serum magnesium. A comparison of the results of analyses by the present procedure and by an automated fluorometric procedure on identical serum specimens shows excellent agreement.

RECENT REPORTS from this laboratory described the application of automated atomic absorption spectrophotometry (AAS) to the determination of calcium in biologic fluids (*1*). In the present communication, the extension of this new analytic technic to the determination of serum magnesium is presented.

Materials and Methods

Instrumentation

The sample presentation modules, atomic absorption spectrophotometric and other instrumental components used were described in the first paper of this series (*1*). In the present procedure, a hollow cathode magnesium lamp was the source and the monochromator grating was adjusted to transmit at 285.2 mμ.

Reagents

The serum diluent and dialyzate recipient solutions were described in the previous paper (*1*).

Standards Standard magnesium solutions containing 0.41-3.28 mEq./L. are prepared as described in a previous publication (*2*).

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Flow Diagram

The flow diagram (Fig. 1) is identical with the one described previously (*1*) but is included here for convenience.

Operating Procedure

The spectrophotometer and the hollow cathode lamp are placed into operation and the readout meter is connected to the recorder. The

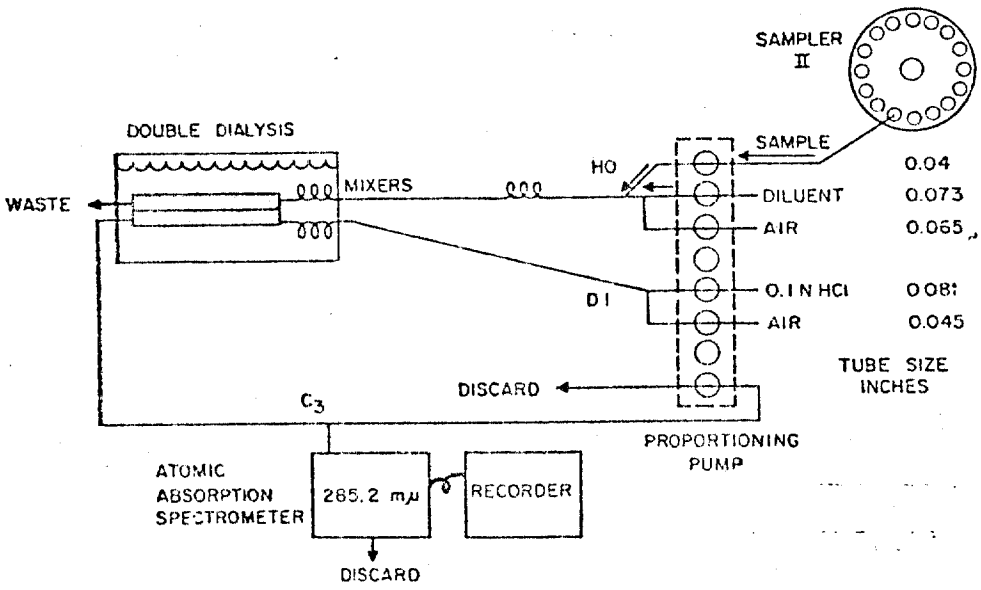

Fig. 1. Serum magnesium by automated AAS. Flow diagram, with dialysis (flow system A).

atomizer-burner is ignited, the fuel and air controls are adjusted to give maximum operating efficiency, and the system is allowed to stabilize for 30 min. In these experiments the lamp current was 4 ma.; the air pressure was 15 psi; acetylene pressure, 2–3 psi; the monochromator was adjusted to peak response (usually at 285.2 mμ) and a 100-μ slit was used.

Diluent and recipient solutions are pumped through the manifold for several minutes (water through the sample line) and the recorder base line is set by first adjusting the readout meter dial to 100% transmittance and then, resetting the recorder zero to the 90 mark on the strip chart. Pumping is continued for a sufficient time to permit baseline stabilization (about 10 min.).

The magnesium standards are sampled in increasing concentration—with the highest concentration immediately followed by the lowest concentration—to determine the degree of interaction. The serum speci-

5.5 ml. of 1 N NaOH and diluted to 100 ml. with water. This solution is stable for at least 2 months, if refrigerated and protected from light (3).

2. *Working reagent A, 0.0025 M* 10 ml. of stock reagent solution is diluted to 200 ml. with 0.1 M Tris buffer, and the solution is brought to pH 7.0 with a few drops of 5 N HCl. This reagent is used in the dialysis procedure (Fig. 1).

3. *Working reagent B, 0.0075 M* 30 ml. of stock reagent solution is diluted to 200 ml. with 0.1 M Tris buffer and brought to pH 7.0 with a few drops of 5 N HCl. This reagent is used in the direct procedure (Fig. 2).

These solutions are stable with refrigeration for at least 2 weeks.

Ethylene bis(oxyethylenenitrilo)tetraacetic acid (EGTA) solution, 0.01 M 3.80 gm. of EGTA (K and K Laboratories, Inc.) is dissolved in 1 L. of 0.1 M Tris buffer, and the pH is adjusted to 8.0 with 5 N HCl.

EGTA solution, 0.002 M 200 ml. of the 0.01 M EGTA solution is diluted to 1 L with 0.1 M Tris buffer, pH 8.0.

Magnesium standards Standard solutions are prepared from pure, oven-dried (800°) magnesium oxide, as described by Schachter (5). Alternatively, 0.3528 gm. of magnesium acetate tetrahydrate crystals, taken from a freshly opened bottle of reagent grade material, is dissolved in water containing a few drops of 5 N HCl and diluted to 1 L. This is equivalent to 4.0 mg. Mg^{++}/100 ml. (3.28 mEq./L.). Dilu-

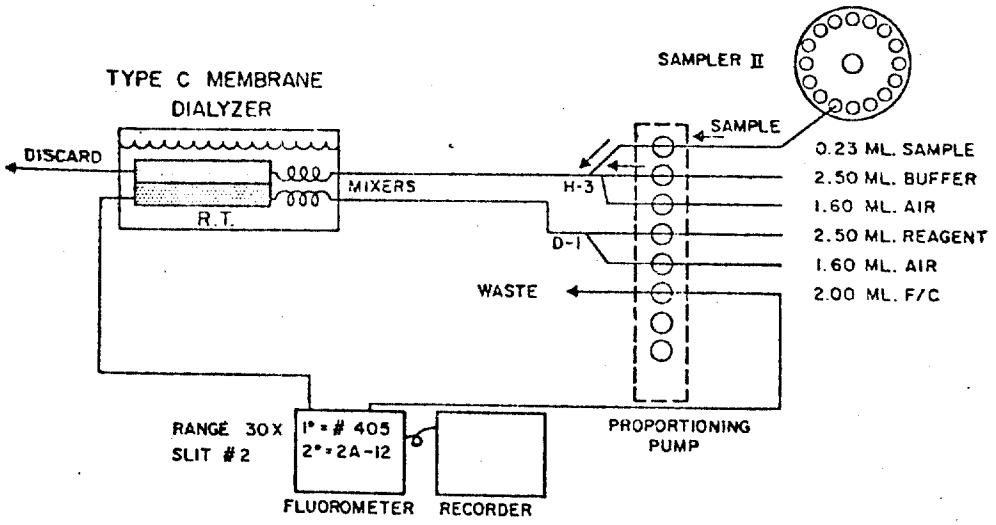

Fig. 1. Flow diagram for fluorometric serum magnesium determination with dialysis. Type C cupriphane membrane is used.

mens are then sampled. All standards are resampled at intervals and at the end of the analysis. A calibration curve is constructed by plotting the mean recorder readings for each standard solution against concentration on semilogarithm ruled graph paper. The magnesium concentration in serum specimens is determined by reference to this plot.

Results

Recordings of a typical calibration curve are shown in Fig. 2. The response is linear to about 3.0 mEq. Mg^{++} per liter. At 3.28 mEq./L.

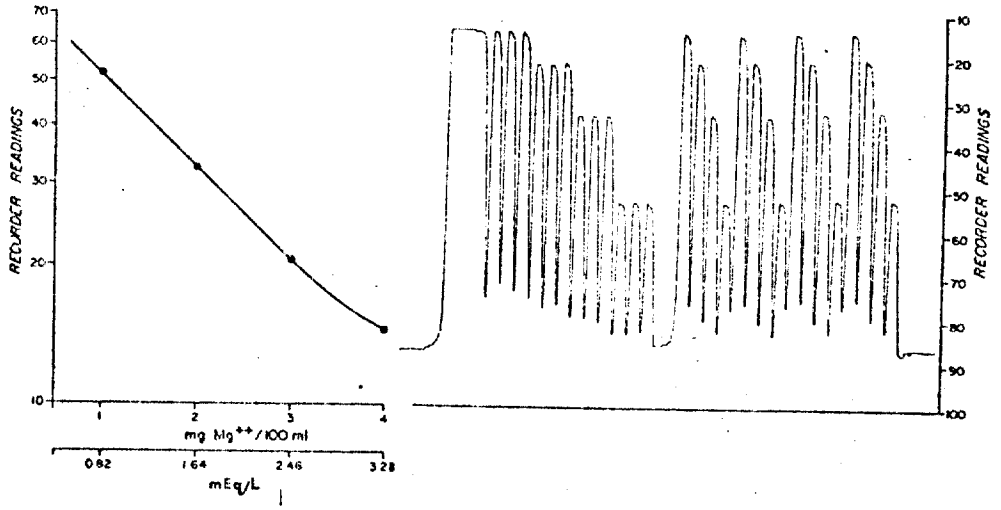

Fig. 2. Serum magnesium by automated AAS. Typical calibration curve and recordings used to plot curve. Recordings of magnesium standard solutions sampled first in increasing concentration, then successively in triplicate, and finally by continuous sampling of standard (4.0 mg. Mg^{++} per 100 ml.).

(4.0 mg. Mg^{++} per 100 ml.) a slight reduction in linearity (mean, 4%) was observed. Also shown are the recordings of standard magnesium solutions sampled successively in increasing order, each standard in triplicate followed by the continuous sampling of the 3.28 mEq./L. magnesium standard. There was rarely more than 1% interaction when a 0.82 mEq./L. magnesium standard immediately followed a 3.28 mEq./L. standard. Replication of standard solutions in the present procedure also were usually within one scale division on the strip chart, frequently within 0.5 scale division (*1*). One division under the indicated experimental conditions was equivalent to 0.043 mEq. Mg^{++} per liter.

Recovery of added magnesium to serum specimens consistently averaged 102% (range 100.7–103.6%), although in a few experiments up to 105% recovery was realized.

Discussion

The determination of serum magnesium by AAS was examined by several investigators (3–8) who reported generally, that under proper conditions, this technic produced reliable and accurate analyses with a sensitivity that overcame the disadvantages of familiar chemical procedures. The successful development of automated procedures for calcium in biologic fluids prompted an extension of this approach to the determination of serum magnesium.

Magnesium analyses by a simpler flow system (Fig. 3) afforded recordings shown in Fig. 4 and 5. The recorder responses were precise, reproducible, and demonstrated good linearity with concentration.

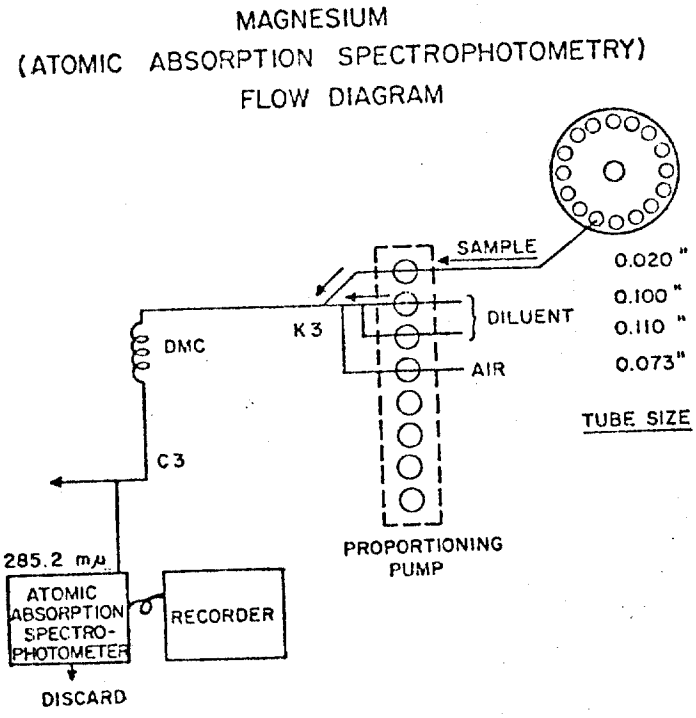

Fig. 3. Serum magnesium by automated AAS. Flow diagram, without dialysis (flow system B).

Serum magnesium analyses obtained by this flow system compared well with manual atomic absorption analyses, and recovery experiments disclosed a mean recovery of 100.5%. The uneven quality of the recording peaks, however, and the occasional recorder base-line drift could not be ascribed wholly to the accumulation of combustion residues of the serum protein or other organic and inorganic materials in the 0.16-ml. sample, causing altered flame characteristics or burner clogging. Furthermore, similar recordings were also produced by aqueous magnesium solutions.

It was decided, therefore, to return to a flow system similar to the one used for serum calcium analyses (*1*). The sample volume was slightly increased to accommodate the requirements of the improved flow system. Over-all stability was achieved since recorder base-line

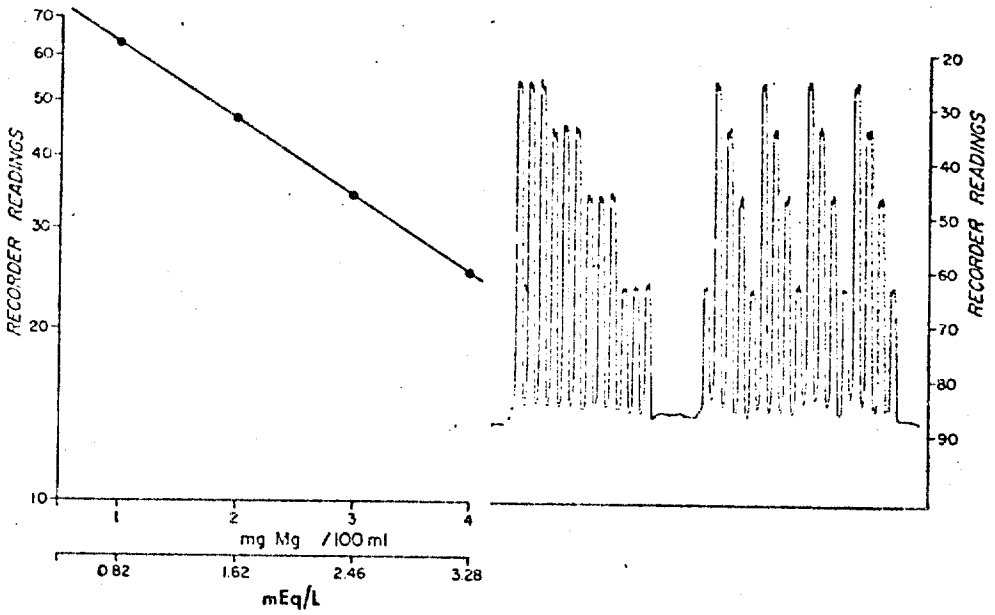

Fig. 4. Serum magnesium by automated AAS. Typical calibration curve and recordings obtained with flow system B. Experimental design similar to that in Fig. 2.

drift now rarely exceeded one scale division. Complemented by the inherently greater sensitivity of magnesium analysis by atomic absorption spectrophotometry, smooth recordings were produced, where each chart scale division was still identical with the response obtained in the nondialyzed flow system. This is illustrated in Fig. 2 and 6.

During the development of the present procedure, specimens and standards were sampled at the 40/hr. rate (2:1, sample:wash). Subsequent experiments disclosed that sampling at the 60/hr. rate (2:1, sample:wash), yielded recordings 97% of the continuous sampling response with no increase in sample interaction (Fig. 7). This is the recommended rate.

Enhancement of magnesium absorption by serum protein was reported by Willis (*3*), who recommended suppression of this interference with Sr^{++}. Stewart *et al.* (*5*) in a later study of physical and chemical effects, found Ca^{++} and SO_4^{--} interference and suggested incorporation of these ions in both serum specimens and standard solutions. No phosphate interference was reported at 6.3-mM levels,

which is approximately double the normal serum level. In the procedure presented in this paper, the acidified lanthanum trichloride diluent solution was retained because its further use in tissue analysis (*9*) and in the analysis of fluids containing larger amounts of phosphate was

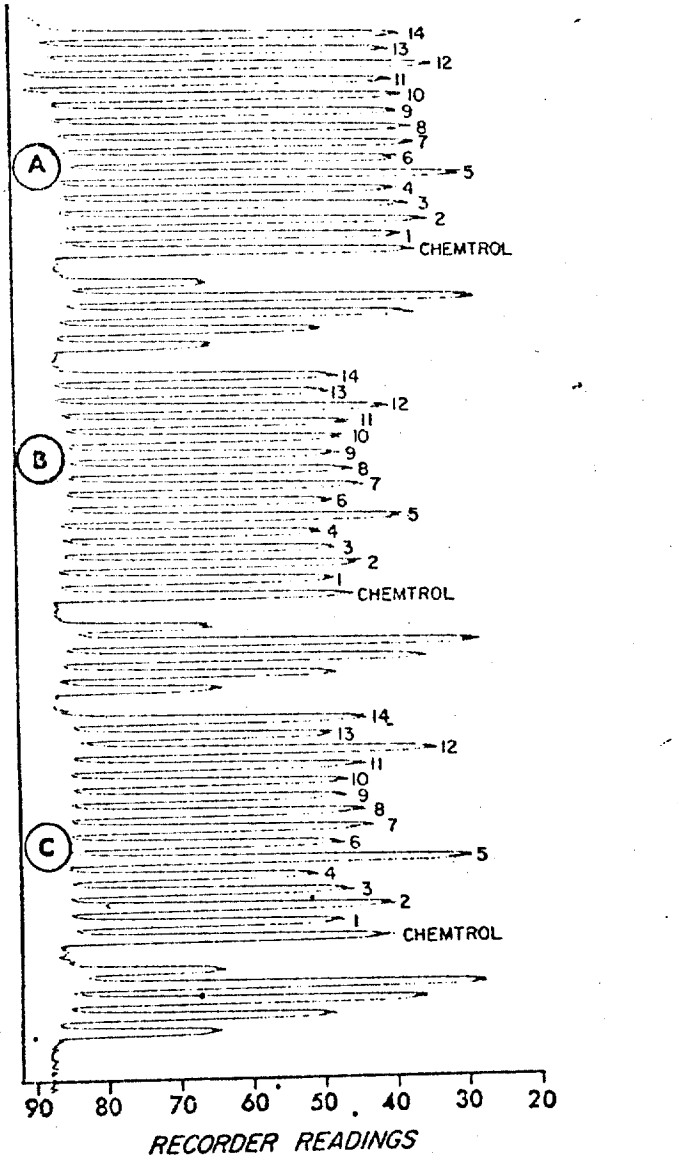

Fig. 5 A, B, C. Serum magnesium by automated AAS. Analyses of 14 serums and commercial control serum (Chemtrol*). A and B. Recovery analyses (flow system B), with serums diluted 1:1 with standards: 4 mg./100 ml., and 2 mg./100 ml., respectively. C. Serum specimens.

contemplated. No experiments were conducted without inclusion of the suppressant in the serum diluent.

Recent disagreements concerning the magnesium concentration in

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normal human serum (*10*) prompted the analysis of a series of serum specimens obtained from healthy human subjects by the present procedure. Twenty of the 24 serums were also analyzed by the automated fluorometric technic recently developed (*2*). The results are given in

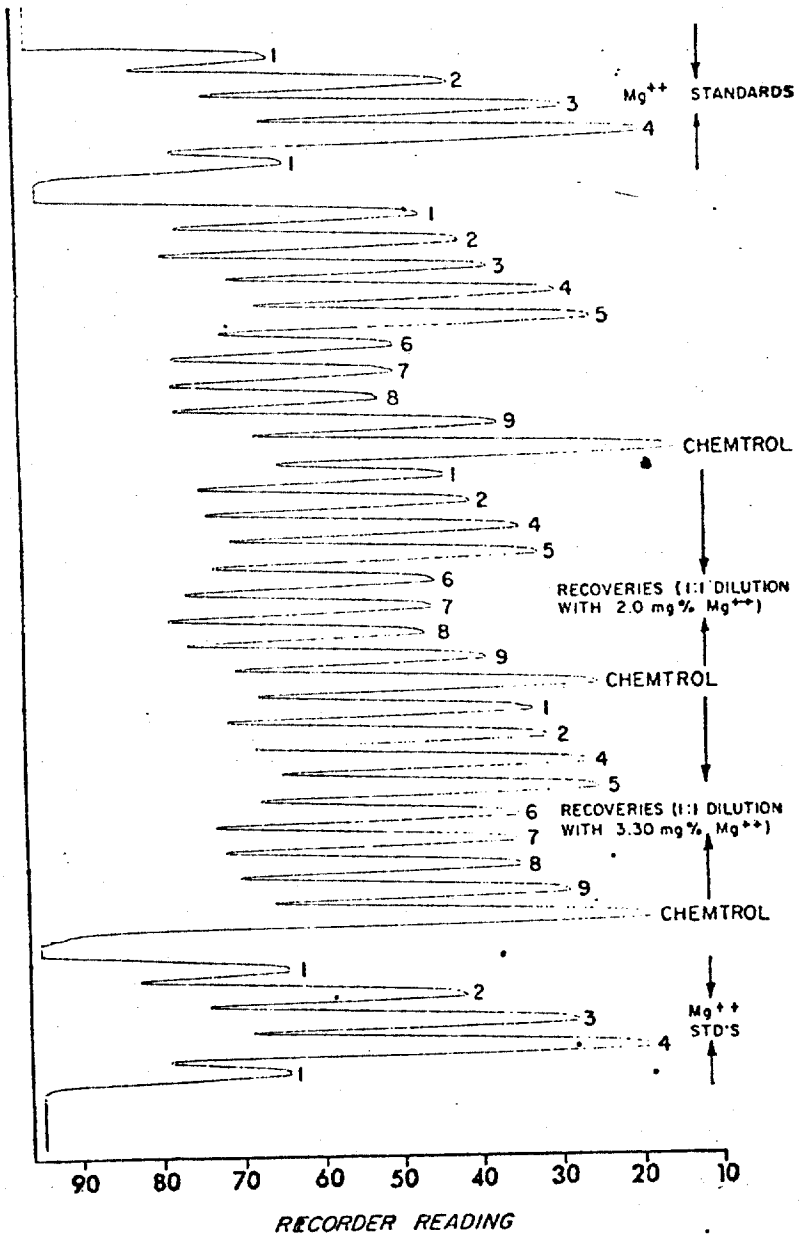

Fig. 6. Serum magnesium by automated AAS. Analysis of 9 serum specimens and commercial control serum by flow system A. Also, two series of recovery analyses.

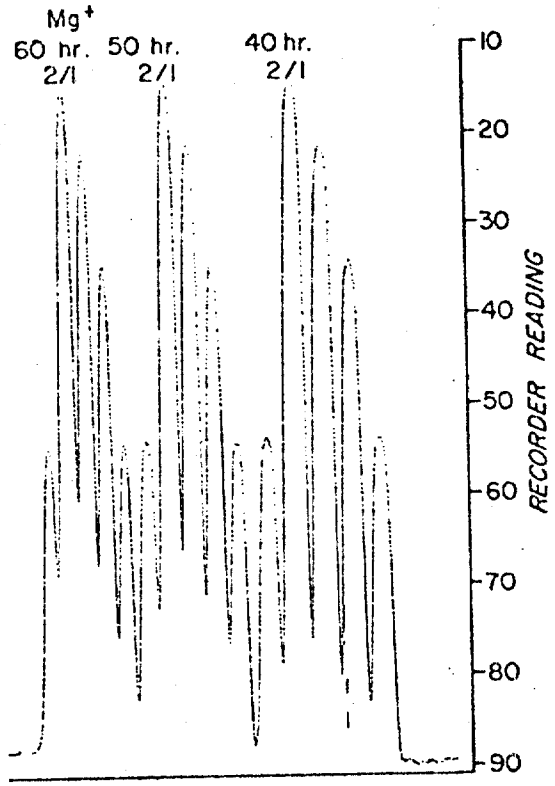

Fig. 7. Serum magnesium by automated AAS. Effect of sampling rate on response.

Table 1. Serum Magnesium Content in Normal Subjects (mEq./L.)

Specimen no.	Methods: AAS	Methods: Fluorometric	Difference
1	1.86	1.79	+ 0.07
2	1.89	1.87	+ 0.02
3	1.66	1.61	+ 0.05
4	1.97	1.92	+ 0.05
5	1.85	1.81	+ 0.04
6	1.84	1.81	+ 0.03
7	1.87	1.93	− 0.06
8	1.88	1.98	− 0.10
9	1.84	1.82	+ 0.02
10	1.72	1.69	+ 0.03
11	1.94	1.95	− 0.01
12	1.60	1.54	+ 0.06
13	2.01	1.98	+ 0.03
14	2.07	2.04	+ 0.03
15	1.56	—	—
16	1.89	—	—
17	1.66	—	—
18	1.49	—	—
19	1.48	1.49	− 0.01
20	1.72	1.69	+ 0.03
21	1.86	1.95	− 0.09
22	1.35	1.43	− 0.08
23	1.35	1.41	− 0.06
24	1.93	1.92	+ 0.01
Mean	1.76	1.78	± 0.04
S. D.	± 0.20	± 0.19	

Table 1. By the atomic absorption procedure the mean serum magnesium concentration was 1.76 mEq./L. (S.D., 0.20), almost identical with the value reported by Stewart *et al.* (*5*) for 100 subjects. This figure is consistent also with the value of approximately 1.7 mEq./L. obtained by diverse methods and cited by Alcock *et al.* (*10*), but lower than the concentration reported by Wacker *et al.* (*11*). By the fluorometric procedure the mean was 1.78 (S.D., 0.19). This is consistent with the serum magnesium concentrations by fluorometry reported by Schachter (*12*) and by Thiers (*13*). The results obtained by the analysis of a more extended series will be presented in another communication.

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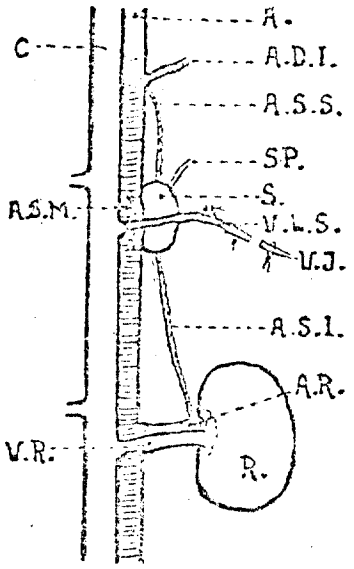

Figure 1. Diagram indicating the anatomical configuration of the suprarenal region where the application of the technique of fistulization of the suprarenal vein can be carried out. At A, aorta; at C, vena cava; at A.D.I., inferior diaphragmatic artery; at S.P., splanchnic; at S, suprarenal; at A.S.M., middle suprarenal artery; at V.L.S., lumbar suprarenal vein; at V.J., jugular vein; at A.S.I., inferior suprarenal artery; at A.R., renal artery; at R, kidney; at V.R., renal vein. Each circulatory arrest caused by constricting the suprarenal vena cava immediately produces the passage of blood into the lumbar suprarenal vein which is artificially elongated by a fragment of the jugular vein.

the excitation of a zone in the hypothalamus which regulates adrenaline secretion.

The experiments performed by one of us in collaboration with R. Saric[4] have made it possible to establish that the volume of the suprarenal venous blood markedly increases in dogs in which the encephalic nerve centers are perfused with blood coming from the same species, which has been made hypoglycemic by insulin administration. The central origin of postinsulin hyperadrenalinemia is found therefore, to be confirmed.

The work on the influence of hypnotics on diuresis has led E.P. Pick and his co-workers to consider that moderate doses of barbiturate derivatives paralyse the thalamic centers in a selective manner. On the other hand, Wauters and one of us[5], have observed the destruction of postinsulin gastric hypersecretion in animals which have received hypnotic doses of sodium barbital.

These results induced us to study whether hypnotic doses of barbital or magnesium sulfate, would modify postinsulin hyperadrenalinemia by particularly paralysing the thalamic centers.

(a) The effect of sodium barbital on postinsulin hyperadrenalinemia

In this series of experiments, we used dogs lightly anesthetised with chloralose then intravenously given 100-150 mg/kg BW of sodium barbital. To quantitatively evaluate adrenaline secretion we used the method of fistulization of the suprarenal vein. Adrenalinemia was evaluated by the comparative study of the inhibiting action on intestinal motility that various samples of blood collected from the lumbar extremity of the artifically extended suprarenal vein, possess. In order to lessen the loss of blood inherent in a permant suprarenal fistulization, it is preferable in place of performing a permanent ligature of the opening

of the suprarenal vena cava, not to induce the venous flow by the suprarenal lumbar vein until after traction of the slack thread placed on the suprarenal vena cava (see the diagram in Figure 1).

By carrying out this operation several minutes before each sampling of suprarenal blood, one avoids the hypotension and the changes of adrenaline secretion owing to the continued bleeding which becomes established at the time of a permanent suprarenal fistulization.

When following this technique we injected chloralosed dogs maintained for 20 minutes under the influence of sodium barbital, with a dose of 0.5-1 unit of insulin per kg BW. Afterwards we carried out the sampling of the suprarenal venous blood the entire 20 minutes in order to keep track of the changes in adrenalinemia in the course of the progressive hypoglycemia released in this way.

Whereas in the control dogs subjected only to the effect of chloralose, a definite hyperadrenalinemia is set-up as soon as glycemia falls below 650 mg/liter (Figure 2), one does not observe any appreciable modification of the adrenaline content of the suprarenal venous blood after administration of sodium barbital (Figure 3).

These results lead one to think that sodium barbital inhibits post-insulin hyperadrenalinemia by decreasing the excitability of the thalamic region.

It is possible therefore that the impediment both to diuresis and to postinsulin hyperadrenalinemia caused by the sodium barbital, could be interpreted as the consequence of a paralysis of the regulatory regions for diuresis located in the thalamic region.

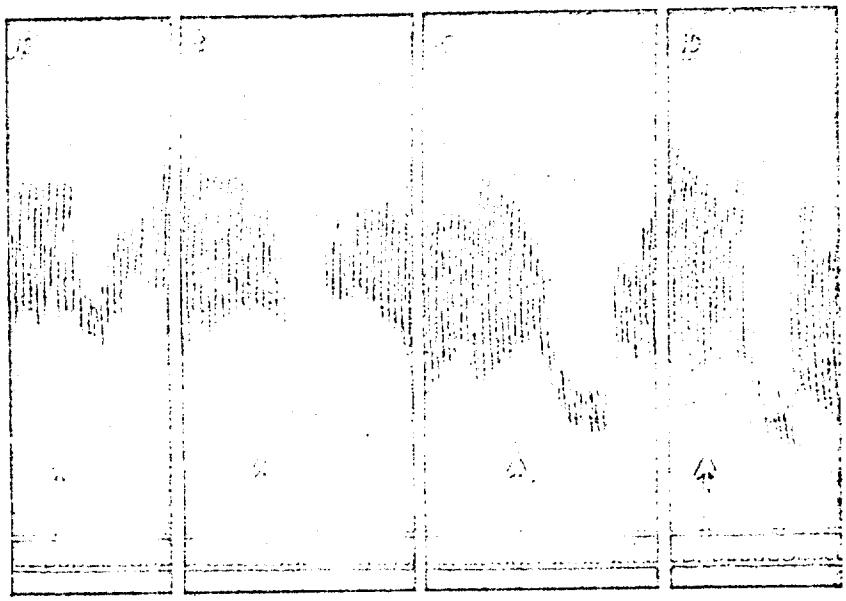

Figure 2. Movements of a rabbits' isolated intestinal loop.

At A, addition of 0.25 cc of normal suprarenal blood.

At B, C, D, addition of the same amount of suprarenal venous blood collected 20, 40, and 80 minutes after the injection of 0.5 unit of insulin/kg.

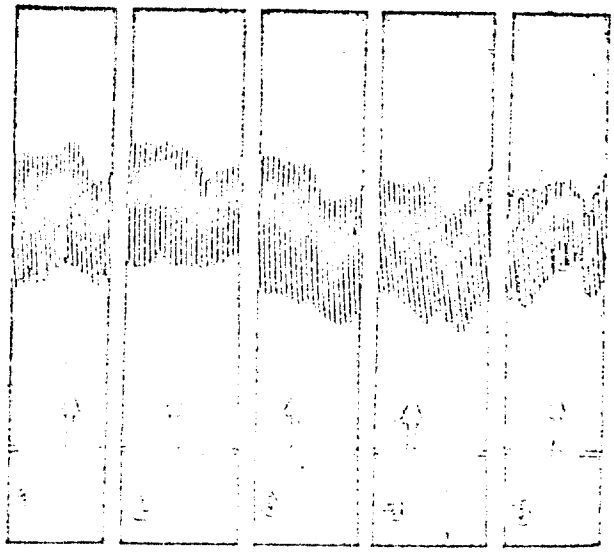

Figure 3. Movements of a rabbits' isolated intestinal loop.

At 1, addition of 0.25 cc of normal suprarenal venous blood.

At 2, 3, 4, and 5, addition of the same quantity of suprarenal venous blood collected 20, 40, 80, and 100 minutes after administration of 0.5 unit of insulin/kg to a chloralosed dog which had received 100 mg/kg BW of sodium barbital.

(b) Effect of Magnesium Sulfate on Postinsulin Hyperadrenalinemia

According to Pick (9) and Yamawaki (10), magnesium ions exert their narcotic effect as a result of their selective fixation on the cerebral peduncle and on the striated body. It seemed of interest to us to study the influence of magnesium sulfate on postinsulin hyperadrenalinemia, whose origin in the central nervous system had been shown by Saric and one of us[4].

By using exactly the same techniques as in the previously described studies, we determined, by the method of fistulization of the suprarenal vein, the effect in the lightly chloralosed dog of intravenously injecting from 100-150 mg/kg BW of magnesium sulfate, on the hyperadrenalinic reaction which accompanies the injection of insulin.

As one observes by examining Figure 4, in the course of the magnesium narcosis no further appreciable change in adrenaline secretion is produced even though the glycemia reaches an amount below 600 mg/liter of blood.

These results are therefore completely comparable to those which we observed when utilising sodium barbital as the hypnotic agent.

The total effect of these verifying experiments is to show that the postinsulin hyperadrenalinic reactions cannot be produced unless the glyco-regulatory and thalamic adrenaline secretory centers retain their functional integrity intact.

(c) Is the Inhibition of Postinsulin Hyperadrenalinemia Peripheral?

The depressive properties of magnesium sulfate have been attributed by Jolyet and Cahours (11) to a purely curare like effect. According to Rabuteau (12), the pharmacological action of this substance is exercised on the smooth musculature itself.

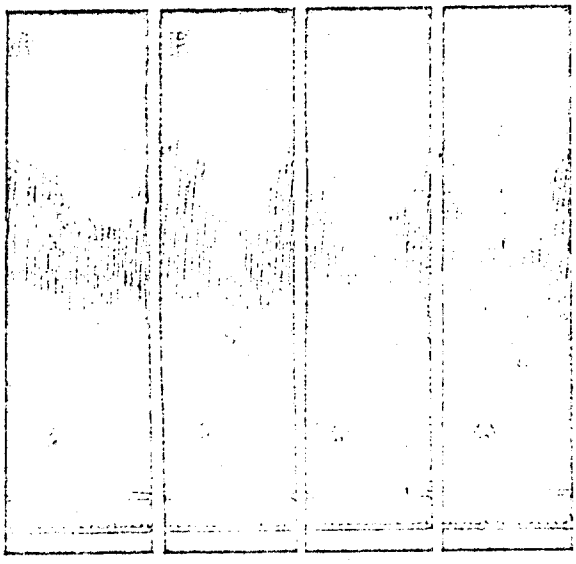

Figure 4. Movements of a rabbit's isolated intestinal loop.
At A, addition of 0.25 cc of normal suprarenal venous blood.
At B, C, D, addition of the same quantity of suprarenal venous blood collected 20, 40, and 80 minutes after the administration of 125 mg/kg of magnesium sulfate.

Per Buiet (13), magnesium sulfate does not unfavorably influence respiration from the fact that it does not exert a curare like effect on the phrenic nerve.

If one is to believe all of these studies and the opinion of Zondek (14), magnesium sulfate manifests a curare-like effect in the first place and only secondarily paralyses the central nervous system.

In a study carried out on the rabbit and the frog, J. Simon (15) has clearly shown that the hypnotic effect of magnesium sulfate already apparent after the administration of the dosage, in no way influences the peripheral nervous system.

In order for the neuromuscular connections to be injured, it is necessary to use very high concentrations of this substance. Therefore it is hardly surprising that Hazard and Wurmser (16) as well as Wodon (17) regard magnesium sulfate as truly curare-like in the sense indicated by Lapicque. In effect, as M. Despres (18) has confirmed, in strong doses this material produces an increase in muscular chronaxy with maintenance of nerve chronaxy.

Because of our concerns, the research of Hazard and Wurmser (19) on the excitability of the sympathetic nervous system under the influence of magnesium sulfate particularly interests us. These authors showed in effect that strong doses of magnesium sulfate increase the cardiac accelerating action of adrenaline and exercise a depressive action on the splanchnic nerve.

But, as we have shown with Saric, postinsulin hyperadrenalinemia results in a hyperexcitability originating in the central and sympathetic nervous systems. Accordingly, before attributing the inhibition of adrenaline secretion which accompanies insulin hypoglycemia to a selective

fixation of Mg ions on the thalamic nervous centers, it is important to make sure that at the dosages used this hypnotic agent does not exert a curare-like effect on the splanchnic nerve endings.

For this reason, we have studied if the effects of electrical excitation at the peripheral end of the splanchnic nerve are appreciably modified when 100 mg of magnesium sulfate is first injected in the animal. The excitation time and the current intensity were strictly kept identical in the course of two successive trials.

Under these conditions, as one can see by examining Figure 5, in the majority of trials if one waits until the blood pressure returns to normal after magnesium sulfate administration, one does not observe reduction of the adrenalinic response from excitation of the splanchnic nerve under the influence of magnesium sulfate. For dosages higher than 150 mg/kg the blood pressure remains depressed for a prolonged time and under these circumstances adrenaline hypertension following nerve stimulation is less pronounced than in the normal state. These observations confirm the results previously obtained by Hazard and Wurmser (16) on the subject of the depressive effect of strong doses of magnesium chloride.

The verifications which we have just made make it possible therefore to conclude that at the doses we used, magnesium sulfate produces post-insulin hyperadrenalinemia by a mechanism which is central in origin. In effect, under these conditions one cannot attribute the suppression of the exaggeration of adrenaline secretion which follows the administration of pancreatic hormone to a peripheral curare-like effect.

General Considerations

The impeding of postinsulin hyperadrenalinemia caused by sodium barbital and magnesium sulfate can be interpreted in different ways. If one agrees

Arch. Int. Pharmacodyn. LXVI(3), 1941

Work from the Laboratory of Pharmacodynamics of Brussels University

Director: Prof. J. LaBarre

A Contribution to the Study of the Paralysing Action of Hypnotics on the Thalamic Centers. II. The Effects of Sodium Barbital and of Magnesium Sulfate on Adrenaline Secretion

Jean LaBarre and Georges Kettenmeyer

(work received April 8, 1941)

Besides the characteristic modifications of the stomach which we have recently pointed out, the hyperexcitable state of the glucose sensitive centers induced by the administration of insulin can involve the hyperactivity of the adrenaline secretory centers.

The position of the regulatory centers of adrenaline secretion has been discussed a great deal. According to Stewart and Rogoff[1], after the section of the dorsal medulla, the adrenaline secretory function is completely destroyed. The authors consider that this observation furnishes the proof of the existence of a dorsal medullary center which regulates suprarenal secretion.

Tournade, Chabrol and Wagner[2], as well as Houssay and Molinelli[3], have proved by the method of suprarenal-jugular anastamosis that all bulbar puncture or faradization of this region results in hyperadrenalemia. These facts argue in favor of the existence of an adrenaline secretory center located in the medulla oblongata.

Furthermore, Houssay and Molinelli have observed that all stimulations carried out on the tubero-infundibular region cause hypertension following

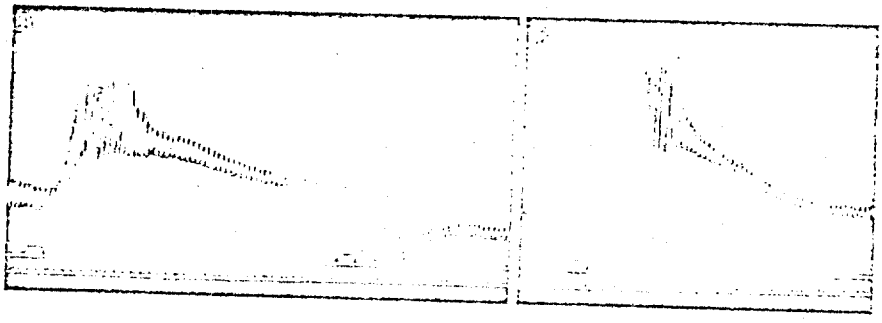

Figure 5. Carotid pressure of a dog in which the splanchnic is excited at E.

Graph A: normal state

Graph B: after administration of 100 mg of $MgSO_4$

The second splanchnic excitation was carried out 15 minutes after the magnesium sulfate injection.

with Houssay and Molinelli (3) that there exist centers important for adrenaline secretion in the thalamic region, one is justified in supposing that these hypnotics paralyse the glucose sensitive and adrenaline secretory centers located in this same region, at the same time. Sodium barbital and magnesium sulfate can, according to a second hypothesis, advanced by Tournade and his co-workers, depress the thalamic region and the bulbar adrenaline secretory centers. Finally, it is possible to imagine that the paralysis alone of the thalamic sensitive zones causes the suppression of all transmission of nervous impulses to the regulatory regions of suprarenal secretion normally connected to the medullary adrenaline secretory centers.

Concerning magnesium sulfate, the inhibition of the exaggeration of postinsulin adrenaline secretion cannot be explained therefore by a curare-like effect of this substance.

The different verifications which we have carried out make it possible to understand better the observations of Winter, Richey and Barbour (20) who have pointed out the reenforcement of the antipyretic effect of phenacetin in dogs subjected to the influence of magnesium salts. In the same way, one should understand what Barbour and Taylor (21) have observed that the addition of magnesium chloride to sodium barbital produces an increase of the narcotic phenomenon.

These various studies lend support to the opinion expressed by Pick according to which magnesium sulfate and the barbiturate derivatives exercise their hypnotic effects by producing a paralysis of the thalamic centers.

SUMMARY

1. Sodium barbital and magnesium sulfate in moderate doses (100-150 mg/kg) produce a complete inhibition of postinsulin hyperadrenalinemia.

2. This phenomenon follows a paralysis of the thalamic centers by these hypnotic agents and is not due to a peripheral curare-like effect.

Arch. int. Pharmacodyn., 1941, LXVI, No 3. 315

TRAVAIL DU LABORATOIRE DE PHARMACODYNAMIE DE L'UNIVERSITÉ DE BRUXELLES. DIRECTEUR : PROF. DR. J. LA BARRE

CONTRIBUTION A L'ÉTUDE DES HYPNOTIQUES A ACTION PARALYSANTE SUR LES CENTRES THALAMIQUES

II. Effets sur barbital sodique et du sulfate magnésique sur l'adrénalinosécrétion.

PAR

JEAN LA BARRE ET GEORGES KETTENMEYER

(Travail reçu le 8-4-1941.)

En dehors des modifications fonctionnelles de l'estomac que nous venons de signaler, l'état d'hyperexcitabilité des centres glycosensibles provoquée par l'administration d'insuline peut entraîner l'hyperactivité des centres adrénalinosécréteurs.

On a beaucoup discuté à propos de la situation des centres régulateurs de l'adrénalino-sécrétion. D'après STEWART et ROGOFF (1), il existe une abolition complète de la fonction adrénalinosécrétoire après section de la moelle dorsale. Cette observation fut considérée par ces auteurs comme apportant la preuve de l'existence d'un centre médullaire dorsal, régulateur de la sécrétion surrénale.

Par la méthode d'anastomose surrénalo-jugulaire, TOURNADE, CHABROL et WAGNER (2), ainsi que HOUSSAY et MOLINELLI (3), ont prouvé que toute piqûre bulbaire ou faradisation de cette région entraîne de l'hyperadrénalinémie. Ces faits plaident en faveur de la présence d'un centre adrénalinosécréteur situé dans la moelle allongée.

Enfin, HOUSSAY et MOLINELLI ont noté que toute stimulation portant sur la région infundibulo-tubérienne détermine de l'hypertension consécutive à l'excitation d'une zone régulatrice de l'adrénalinosécrétion située dans l'hypothalamus.

Les expériences réalisées par l'un de nous en collaboration avec R. SARIC (4) ont permis d'établir que la teneur du sang veineux surrénal s'accroît notablement chez le chien dont les centres nerveux encéphaliques sont perfusés par du sang provenant d'un congénère rendu

hypoglycémique par administration d'insuline. L'origine centrale de l'hyperadrénalinémie postinsulinique se trouverait ainsi confirmée.

L'étude de l'influence des hypnotiques sur la diurèse a amené E. P. PICK et ses collaborateurs, à considérer que les dérivés barbituriques donnés à doses modérées paralysent de façon élective les centres thalamiques. D'autre part, WAUTERS et l'un de nous (5) ont observé une abolition de l'hypersécrétion gastrique postinsulinique chez les animaux qui avaient reçu des doses hypnotiques de barbital sodique.

Ces résultats nous ont engagés à rechercher si des doses hypnotiques de barbital ou de sulfate magnésique, en paralysant plus particulièrement les régions thalamiques, modifieraient l'hyperadrénalinémie postinsulinique.

a) *Action du barbital sodique sur l'hyperadrénalinémie postinsulinique.*

Dans cette série d'expériences, nous avons utilisé des chiens légèrement anesthésiés au chloralose, puis ayant reçu par voie intraveineuse 100 à 150 mg. de barbital sodique (par kg. de poids corporel). Pour évaluer quantitativement la sécrétion adrénalinique, nous avons eu recours à la méthode de fistulisation veineuse surrénale. Par l'étude comparative des effets inhibiteurs sur la motilité intestinale que possèdent divers échantillons de sang recueillis à l'extrémité lombaire de la veine surrénale artificiellement prolongée, on peut évaluer l'adrénalinémie. Afin de diminuer la perte de sang inhérente à une fistulisation surrénale permanente il est préférable au lieu de pratiquer la ligature définitive de l'abouchement cave de la veine surrénale, de ne provoquer l'écoulement veineux par la veine surrénale lombaire qu'après traction sur le fil lâche placé sur la veine surrénale cave (voir schéma de la figure 1).

En effectuant cette manœuvre quelques minutes avant chaque prise de sang surrénal, on évite l'hypotension et les changements de l'adrénalinosécrétion dus à la saignée continue qui s'établit lors d'une fistulisation surrénale permanente.

En utilisant cette technique, nous avons injecté au chien chloralosé et maintenu pendant 20 minutes sous l'influence du barbital sodique, une dose de 0.5 à 1 unité d'insuline (par kg. de poids corporel). Nous avons ensuite effectué des prises de sang veineux surrénal toutes les 20 minutes afin de nous rendre compte des variations de l'adrénalinémie au cours de l'hypoglycémie progressive ainsi déclenchée.

Alors que chez les chiens témoins soumis uniquement à l'influence du chloralose, il s'installe une hyperadrénalinémie nette aussitôt que

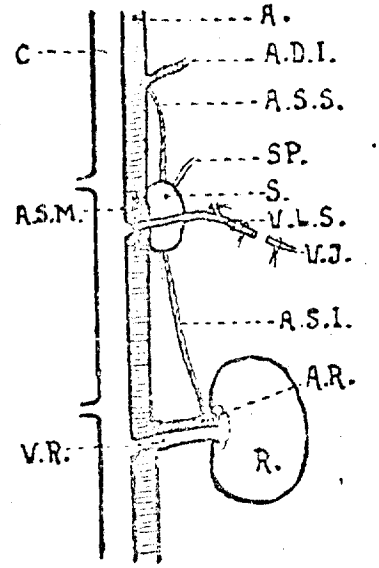

FIGURE 1

Schéma indiquant la configuration anatomique de la région surrénale permettant l'application de la technique de fistulisation veineuse surrénale.

En *A*, aorte; en *C*, veine cave; en *A.D.I.*, artère diaphragmatique inférieure; en *A.S.S.*, artère surrénale supérieure; en *SP*, splanchnique; en *S*, surrénale; en *A.S.M.*, artère surrénale moyenne; en *V.L.S.*, veine lombo-surrénale; en *V.J.*, veine jugulaire; en *A.S.I.*, artère surrénale inférieure; en *A.R.*, artère rénale; en *R*, rein; en *V.R.*, veine rénale.

Tout arrêt circulatoire provoqué par pincement de la veine surrénale cave entraîne immédiatement le passage du sang dans la veine lombo-surrénale artificiellement prolongée par un fragment de veine jugulaire.

la glycémie tombe en dessous de 650 mg. par litre (FIG. 2), on n'observe aucune modification appréciable de la teneur du sang veineux surrénal en adrénaline après l'administration de barbital sodique (FIG. 3).

Ces résultats permettent de penser que le barbital sodique inhibe l'hyperadrénalinémie postinsulinique en diminuant l'excitabilité de la région thalamique.

Ces observations peuvent être mises en relation avec les constatations de WALTON (6) suivant lesquelles la diurèse aqueuse se trouve également fortement atténuée sous l'influence des dérivés barbituriques. Cette action inhibitrice n'existe pas d'après BONSMANN (7) et BERGWALL (8) chez les animaux traités par des anesthésiques corticaux tels que le chloral et la paraldéhyde.

Il est donc possible que l'entrave apportée par le barbital sodique tant à la diurèse qu'à l'hyperadrénalinémie postinsulinique, puisse s'interpréter comme la conséquence d'une paralysie des centres adrénalinosécréteurs et des régions régulatrices de la diurèse situés dans la région thalamique.

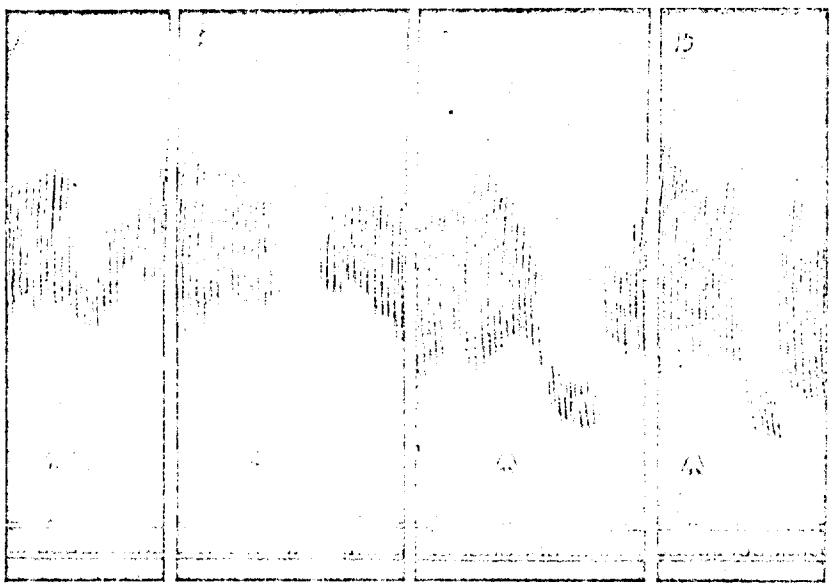

FIGURE 2

Mouvements d'une anse intestinale isolée de lapin.

En *A*, addition de 0.25 cc. de sang veineux surrénal normal.

En *B*, *C*, *D*, addition de la même quantité de sang veineux surrénal recueilli 20, 40 et 80 minutes après l'injection de 0.5 unité d'insuline (par kg.).

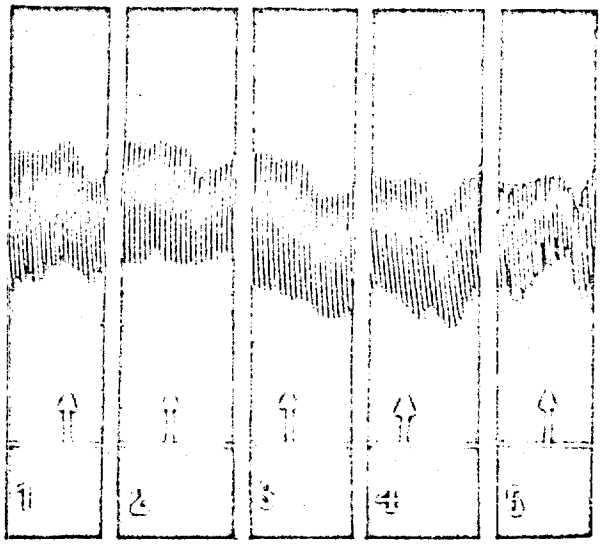

FIGURE 3

Mouvements d'une anse intestinale isolée de lapin.

En 1, addition de 0.25 cc. de sang veineux surrénal normal.

En 2, 3, 4 et 5, addition de la même quantité de sang veineux surrénal recueilli 20, 40, 80, 100 minutes après l'administration de 0.5 unité d'insuline par kg. à un chien chloralosé, ayant reçu 100 mg. de barbital sodique par kg. de poids corporal.

b) *Action du sulfate magnésique sur l'hyperadrénalinémie postinsulinique.*

D'après PICK (9) et YAMAWAKI (10), les ions Mg auraient une action narcotisante par suite de leur fixation élective sur le pédoncule cérébral et sur le corps strié. Il nous a paru intéressant de rechercher l'influence du sulfate magnésique sur l'hyperadrénalinémie postinsulinique dont l'origine nerveuse centrale avait été démontrée par SARIC et l'un de nous (4).

En utilisant exactement la même technique que pour les essais précédemment décrits, nous avons, chez le chien légèrement chloralosé, apprécié, par la méthode de fistulisation veineuse surrénale, les effets de l'injection intraveineuse de 100 à 150 mg. par kg. de poids corporel de sulfate magnésique sur la réaction hyperadrénalinique qui accompagne l'injection d'insuline.

Ainsi qu'on le note à l'examen de la figure 4 au cours de la narcose magnésienne, il ne se produit plus aucune variation appréciable de l'adrénalinosécrétion même lorsque la glycémie atteint des chiffres inférieurs à 600 mg. par litre de sang.

Ces résultats sont donc entièrement comparables à ceux que nous avons observés en utilisant le barbital sodique comme agent hypnotique.

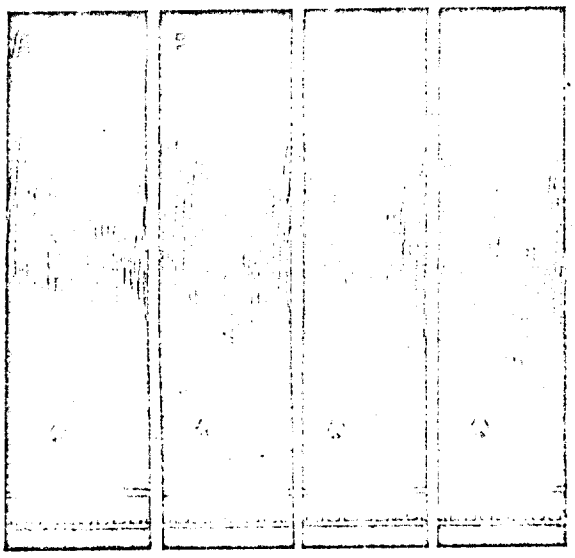

FIGURE 4

Mouvements d'une anse intestinale isolée de lapin.
En *A*, addition de 0.25 cc. de sang veineux surrénal normal.
En *B*, *C*, *D*, addition de la même quantité de sang veineux surrénal recueilli 20, 40 et 80 minutes après l'administration de 125 mg. de sulfate magnésique (par kg.).

L'ensemble de ces constatations montre que les réactions hyperadrénaliniques postinsuliniques ne peuvent se produire que lorsque les centres glycorégulateurs et adrénalinosécréteurs thalamiques conservent leur intégrité fonctionnelle complète.

c) *L'inhibition de l'hyperadrénalinémie postinsulinique est-elle d'origine périphérique ?*

Les propriétés dépressives du sulfate de magnésie avait été attribuées par JOLYET et CAHOURS (11) à un effet purement curarisant. D'après RABUTEAU (12), l'action pharmacologique de cette substance s'exercerait sur la musculature lisse elle-même.

Pour BINET (13), le sulfate magnésique n'influencerait pas défavorablement la respiration du fait qu'il n'exerce pas d'action curarisante sur le nerf phrénique.

Si l'on s'en rapporte à l'ensemble de ces travaux et à l'opinion de ZONDEK (14), le sulfate magnésique manifesterait en premier lieu une action curarisante et ne paralyserait que secondairement le système nerveux central.

Dans une étude faite chez le lapin et chez la grenouille, J. SIMON (15) a démontré nettement que l'action hypnotique du sulfate magnésique apparaît déjà après l'administration de doses n'influençant nullement le système nerveux périphérique. Pour que les connexions neuromusculaires soient atteintes, il faut employer des concentrations très élevées de ce produit. Aussi n'est-il guère étonnant que HAZARD et WURMSER (16) ainsi que WODON (17) considèrent le sulfate magnésique comme un curarisant vrai dans le sens indiqué par LAPICQUE. En effet, ce produit entraîne, à fortes doses comme M. DESPRES (18) l'a confirmé, une augmentation de la chronaxie musculaire avec maintien de la chronaxie nerveuse.

Pour ce qui nous concerne, les recherches de HAZARD et WURMSER (19) sur l'excitabilité du sympathique sous l'influence du sulfate magnésique nous intéressent tout spécialement. Ces auteurs montrent en effet que les fortes doses de sulfate magnésique augmentent l'action cardio accélératrice de l'adrénaline et exercent une action dépressive sur le nerf splanchnique.

Or, l'hyperadrénalinémie postinsulinique résulte, comme nous l'avons montré avec SARIC, d'une hyperexcitabilité d'origine centrale et sympathique. Aussi, avant d'attribuer l'inhibition sécrétoire adrénalinique qui accompagne l'hypoglycémie insulinique à une fixation élective des ions Mg sur les centres nerveux thalamiques, importait-il de s'assurer

qu'aux doses utilisées, cet agent hypnotique n'exerçait pas d'action curarisante sur les terminaisons splanchniques.

Pour cela, nous avons recherché si les effets de l'excitation électrique du bout périphérique du splanchnique se trouvent sensiblement modifiés lorsqu'on injecte au préalable à l'animal 100 mg. de sulfate magnésique (par kg.). Le temps d'excitation et l'intensité du courant sont maintenus rigoureusement identiques au cours des deux essais successifs.

Dans ces conditions, ainsi qu'on peut s'en rendre compte à l'examen de la figure 5, dans la majorité des essais si l'on attend que la pression sanguine soit revenue à la normale après l'administration de sulfate magnésique, on ne note pas, sous l'influence du sulfate magnésique, de diminution de la réponse adrénalinique à l'excitation du splanchnique. Pour des doses supérieures à 150 mg. (par kg.), la pression sanguine reste abaissée pendant un temps prolongé et dans ces circonstances l'hypertension adrénalinique consécutive à la stimulation nerveuse est moins accusée qu'à l'état normal. Ces observations confirment les résultats antérieurement obtenus par HAZARD et WUHRMSER (16) au sujet de l'action dépressive de fortes doses de chlorure magnésique.

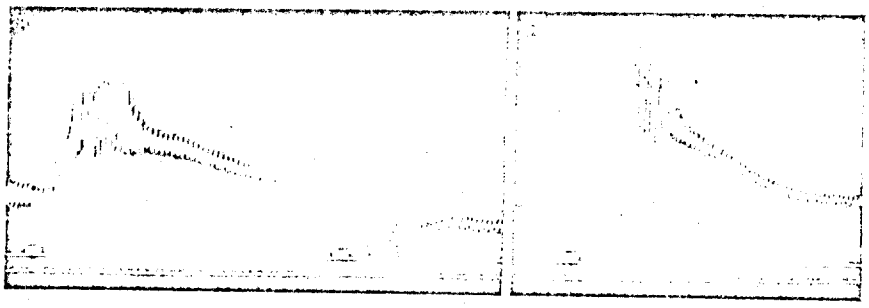

FIGURE 5

Pression carotidienne d'un chien dont on excite le splanchnique en *E*.
Tracé *A* : à l'état normal.
Tracé *B* : après administration de 100 mg. de SO_4 Mg.
La seconde excitation du splanchnique a été pratiquée 15 minutes après l'injection de sulfate magnésique.

Les constatations que nous venons de faire nous permettent donc de conclure qu'aux doses que nous avons utilisées, le sulfate magnésique entraîne l'inhibition de l'hyperadrénalinémie postinsulinique par un mécanisme d'origine centrale. En effet, dans les conditions où nous nous sommes placés, on ne peut attribuer la suppression de l'exagération sécrétoire adrénalinique qui suit l'administration de l'hormone pancréatique à un effet curarisant périphérique.

Considérations générales

L'entrave apportée par le barbital sodique et le sulfate magnésique à l'hyperadrénalinémie postinsulinique peut s'interpréter de différentes façons. Si l'on admet avec Houssay et Molinelli (3) l'existence de centres importants de l'adrénalino-sécrétion dans la région thalamique, on est autorisé à supposer que ces hypnotiques paralysent à la fois les centres glycosensibles et adrénalinosécrétoires situés dans cette même région. Le barbital sodique et le sulfate magnésique pouvaient, selon une deuxième hypothèse, déprimer la région thalamique et les centres adrénalinosécréteurs bulbaires, admis par Tournade et ses collaborateurs. Enfin il serait possible de concevoir que la seule paralysie des zones sensibles thalamiques entraine la suppression de toute transmission d'influx nerveux vers des régions régulatrices de la sécrétion surrénale normalement en relation avec les centres adrénalinosécréteurs médullaires.

Pour ce qui concerne le sulfate magnésique, l'inhibition de l'exagération postinsulinique de l'adrénalinosécrétion ne peut donc pas s'expliquer par une action curarisante de cette substance.

Les différentes constatations que nous avons faites permettent de mieux comprendre les observations de Winter, Richey et Barbour (20) qui ont signalé le renforcement de l'action antipyrétique de la phénacétine chez les chiens soumis à l'influence des sels magnésiques. De même on comprendra que Barbour et Taylor (21) aient noté que l'adjonction de chlorure magnésique au barbital sodique produit une augmentation des phénomènes narcotiques.

Ces divers travaux viennent à l'appui de l'opinion émise par Pick et suivant laquelle le sulfate magnésique et les dérivés barbituriques exercent leurs effets hypnotiques en entraînant une paralysie des centres thalamiques.

Résumé

1. Le barbital sodique et le sulfate magnésique produisent déjà à doses modérées (100 à 150 mg. par kg.) une inhibition complète de l'hyperadrénalinémie postinsulinique.

2. Ce phénomène est consécutif à une paralysie des centres thalamiques par ces agents hypnotiques et non à une action curarisante périphérique.

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FRENCH TRANSLATION

The effect of Colloidal Magnesium Hydroxide and Sodium Thiosulfate on White Blood Corpuscles

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Compt. Rend. Soc. de Biol. 103: 366-368, 1930

We consider it unnecessary to explain here the history of our present understanding of phagocytosis; we only mention the name of Metchnikoff who first determined the details of this cellular defense against microbes. Until the work of Hamburger and his school, there have not been systematic studies on the influence that various medicinals can have on this biological phenomenon. This question was enlarged in 1915 by Delbet's discovery[1], especially important from a practical point of view, of the activating action of magnesium chloride on phagocytosis. It was Delbet, who first conceived the importance of magnesium in the animal organism and the recent account of his research confirms this preceding discussion. Magnesium can exert an apparent effect on the living cell which this author denoted with the term of "cytophylactic effect", as for the white blood cells, magnesium chloride causes an increase of phagocytosis, which in vivo can reach 300 percent of the normal level.

It appeared interesting to us to investigate if a similar effect takes place with another magnesium compound, colloidal magnesium hydroxide, whose therapeutic use, under the name Polysane, is particularly recommended by Becka[2]; we also have studied another chemotherapeutic agent, sodium thiosulfate, which recently has become the object of investigations by

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1 Monde Medical, 1929, p. 969. See also C. R. de l'Acad. des Sc., 161:268, 1915.
2 Casopis lekarn ceskych 1927, nos. 22 and 30.

Kabelik[3]. We will now give the results which we have obtained with these two substances in vitro. We have followed the same experimental technique as Hamburger and Delbet. We used white corpuscles from the venous blood of the horse. The blood samples were made in test tubes containing 50 cc of an isotonic citrate solution as an anticoagulant: 0.7% sodium chloride and 1.1% sodium citrate; this amount is enough for 150 cc of blood. Once the white cells were separated and washed we obtained a thick suspension, containing for the most part polynuclear cells. Two cc of this suspension, 2 cc of the solutions being studied and finally 0.25 cc of a B. coli culture in peptone containing broth, more than 24 hours old, was distributed in tubes. Two tubes to which no colloidal hydroxide or sodium thiosulfate were added acted as controls. All the tubes were put in the oven for 10 to 20 minutes; then by means of Pasteur pipettes, slides were made which were stained with Loeffler's alkaline blue. At every concentration of colloidal magnesium hydroxide or sodium thiosulfate (N/10, N/100, N/1,000, N/10,000) we counted 300 polynuclear cells and we determined the number of bacteria which they phagocytised. The accompanying table shows the results we obtained. The numbers indicate the phagocytoses per 100 leucocytes.

Colloidal magnesium hydroxide and sodium thiosulfate are thus shown to be strong stimulators of phagocytosis in vitro; the increase can reach 250 percent in relation to the normal. The intensity of the activity of these two substances is very close; however, it appears to be a little more marked

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[3] Ceska dermatologie 1.5, nos. 3-5.

Experiment	Substance studied	Concentration of the solution				
		Control	N/10	N/100	N/1000	N/10000
1	$Mg(OH)_2$	---	30	49	42	57
	$Na_2S_2O_3$	20	56	33	42	64
2	$Mg(OH)_2$	---	46	164	210	176
	$Na_2S_2O_3$	90	70	219	194	207
3	$Mg(OH)_2$	---	29	51	59	66
	$Na_2S_2O_3$	42	34	54	64	63
4	$Mg(OH)_2$	---	26	53	59	59
	$Na_2S_2O_3$	25	50	58	61	53
5	$Mg(OH)_2$	---	---	100	164	140
	$Na_2S_2O_3$	65	60	144	170	120

for the sodium thiosulfate. The phagocytosic activity has its maximum at the concentrations of the solutions which range between N/1000 to N/10,000; on the other hand, the concentration N/10 weakens or even suppresses the activity of the polynuclear cells.

(Pharmacology Institute of the Veterinary School)

Action de l'hydroxyde colloïdal de magnésie et du thiosulfate de soude sur les globules blancs,

par J. Lebduska et F. Cervinka.

Nous croyons inutile de développer ici l'historique de nos connaissances actuelles sur la phagocytose : citons seulement le nom de Metchnikoff qui a le premier déterminé les circonstances de cette défense cellulaire contre les microbes, depuis lors, aux travaux de Hamburger et de son école, il n'y a pas eu d'études systématiques sur l'influence que peuvent exercer les divers médicaments sur ce phénomène biologique. Cette question a été remise en 1913 par la découverte de Delbet (1), importante surtout au point de vue pratique, sur l'action activante du chlorure de magnésium sur la phagocytose. C'est Delbet qui, le premier, conçut l'importance du magnésium dans l'économie animale et l'exposé récent de ses recherches confirme ses vues précédentes. Le magnésium exercerait sur la cellule vivante une action manifeste que cet auteur désigne du terme d'« action cytophylactique ». Quant aux globules blancs, le chlorure de magnésium amène un

(1) *Monde médical*, 1919, p. 369. Voir aussi *C. R. de l'Acad. des sc.*, 1913, 1914, p. 205.

augmentation de la phagocytose, qui peut atteindre *in vitro* 300 p. 100 du taux normal.

Il nous a paru intéressant de rechercher si une action identique se manifeste avec un autre composé magnésien : l'hydroxyde colloïdal de Mg, dont l'emploi en thérapeutique, sous le nom de « Polysane », a été préconisé surtout par Becka (2) ; nous avons étudié aussi un autre agent chimiothérapique, le thiosulfate de soude, qui a fait, dans ces derniers temps, l'objet de recherches de la part de Kabelik (3). Nous exposerons ici les résultats que nous avons obtenus avec ces deux substances *in vitro*. Nous avons suivi la même technique expérimentale qu'Hamburger et que Delbet. Nous avons utilisé les globules blancs provenant du sang veineux du Cheval. Les prélèvements de sang ont été faits dans des éprouvettes contenant comme anticoagulant 50 c.c. d'une solution isotonique citratée : 0,7 p. 100 de chlorure de sodium et 1,1 p. 100 de citrate de soude ; cette quantité suffisait pour 150 c.c. du sang. Une fois les globules blancs isolés et lavés, nous avons obtenu une suspension épaisse, contenant en majeure partie des polynucléaires. On a distribué dans des tubes 2 c.c. de cette suspension, 2 c.c. des solutions à étudier et enfin 0,25 c.c. d'une culture de *B. coli* en bouillon peptoné, âgée de 24 heures.

Expériences	Substance étudiée	Témoin	Concentration de la solution N/10	N/100	N/1.000	N/10.000
I	$Mg(OH)^2$	20	35	40	32	27
	$Na^2S^2O^3$		56	33	47	64
II	$Mg(OH)^2$	60	46	164	210	170
	$Na^2S^2O^3$		70	209	194	207
III	$Mg(OH)^2$	40	29	51	59	60
	$Na^2S^2O^3$		34	54	64	65
IV	$Mg(OH)^2$	25	26	52	54	50
	$Na^2S^2O^3$		50	58	64	56
V	$Mg(OH)^2$	65		150	165	130
	$Na^2S^2O^3$		60	144	170	170

plus. Deux tubes n'ayant reçu aucune addition d'hydroxyde colloïdal de magnésie ou de thiosulfate de soude servaient de témoins. Tous les tubes ont été portés pendant 10 à 20 minutes à l'étuve ; puis, à l'aide de pipettes de Pasteur, on a fait des frottis qu'on colorait au bleu alcalin de Loeffler. Pour chaque solution d'hydroxyde colloïdal de magnésie et de thiosulfate de soude (N/10, N/100, N/1.000, N/10.000), nous avons [illegible]

(2) *Gazeta lekarska czeska*, 1928, n^os 22 et 23.

(3) *Česka dermatologie*, I, 5, n^os 3-5.

polynucléaires et nous avons déterminé le nombre des Bactéries qu'ils ont phagocytées. Le tableau ci-joint expose les résultats que nous avons obtenus : les chiffres indiquent les phagocytoses p. 100 leucocytes.

L'hydroxyde colloïdal de magnésie et le thiosulfate de soude se sont donc révélés comme des stimulants puissants de la phagocytose *in vitro* ; l'augmentation peut atteindre 250 p. 100 par rapport à la normale. L'intensité d'action des deux substances est très voisine ; elle semble être cependant un peu plus marquée pour le thiosulfate de soude. L'activité de la phagocytose a son maximum pour les concentrations des solutions variant de N/1.000 à N/10.000 ; par contre la concentration N/10 peut diminuer ou même supprimer l'activité des polynucléaires.

(*Institut de pharmacologie de l'École vétérinaire.*)

Nature 198(4883):1058-1060; 1963

PARATHYROID HORMONE AND MAGNESIUM HOMOEOSTASIS

By Dr. I. MacINTYRE, S. BOSS and V. A. TROUGHTON
Postgraduate Medical School, London, W.12

SEVERAL groups of workers[1-3] have claimed that parathyroid hormone reduces calcium excretion in the urine, although Gordan[4] has shown that this is difficult to demonstrate in parathyroid disorders. The situation has been further complicated by the recent report of Widrow and Levinsky[5] that, while parathyroid extract reduces calcium clearance in dogs, no such effect is demonstrable with pure bovine parathyroid hormone.

Dr. Howard Rasmussen was kind enough to supply us with some of his pure parathyroid hormone of potency 2,000 units/mg and we have used this to study the effect of the hormone on the urinary excretion of the divalent cations, magnesium and calcium.

Male 150-gm parathyroidectomized rats were used. The techniques for immobilization and urine collection were those described by Cotlove[6]. A needle was inserted into the tail vein and an infusion at 3 ml./h given using an Auto Analyser pump and micro-tubing. The infusion solution contained: glucose 44 g/l.; sodium chloride 19 m.equiv./l.; calcium lactate 10 m.equiv./l.; magnesium chloride 10 m.equiv./l.; sodium heparin 0·1 g/l. Calcium and magnesium were estimated flame spectrophotometrically with the Zeiss spectrophotometer *PMM12*. The infusions were started at 5 p.m. and continued overnight and throughout the following day, during which stable excretion values for calcium, magnesium, creatinine and phosphate were obtained (Fig. 1). In preliminary experiments, addition of 20 units of parathyroid extract (Lilly) to the infusion over a period of 1 h produced a profound fall in magnesium and calcium excretion with no effect on creatinine excretion (Table 1 and Fig. 2).

Table 1. Effect of Parathyroid Extract on Excretion Rates

	Before	After
Mg (μequiv./min)	0·57 (0·61)	0·25 (0·59)
Ca (μequiv./min)	0·18 (0·17)	0·02 (0·17)
Creatinine (μgm/min)	5·4	5·1

The figures in brackets are excretion rates of the control group of rats at corresponding times to the experimental group. Analysis of variance shows that the falls in magnesium and calcium are highly significant ($P < 0·01$).

More detailed studies were now carried out using the pure hormone. The same striking effects were obtained. Fig. 3 shows the effect of infusing 10 μg of pure hormone. Three points should be noticed: there is a marked fall in magnesium and calcium excretion; the creatinine excretion is unchanged; the greatest effects on calcium and magnesium excretion are synchronous with that on phosphate excretion.

An effect was observed on magnesium with as little as 1 μg. Fig. 4 shows the results of 10 infusions and suggests that there is a relationship between dose and effect. These experiments show conclusively that parathyroid

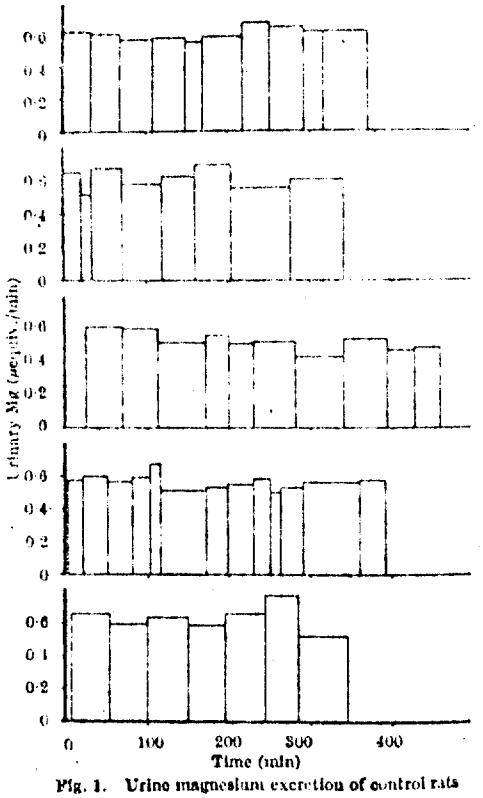

Fig. 1. Urine magnesium excretion of control rats

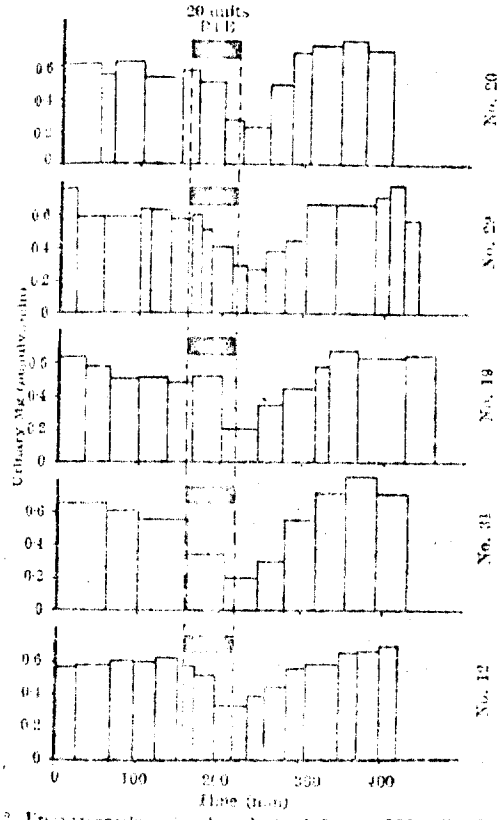

Fig. 2. Urine magnesium excreted during infusion of 20 units of parathyroid extract (Lilly)

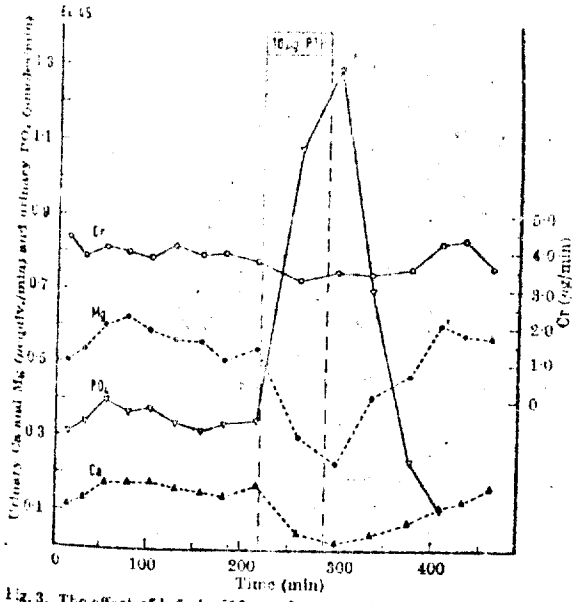

Fig. 3. The effect of infusing 10 μg of pure bovine parathyroid hormone

hormone causes marked conservation of urine magnesium and calcium. It seems likely that this is a direct renal effect and that it is due to a change in the tubular handling of these cations.

Plasma magnesium is maintained by the body within very narrow limits by some unknown homœostatic mechanism. We suggest that this mechanism is the variation in secretion of parathyroid hormone in response to changes of plasma magnesium. This hypothesis is illustrated in Fig. 5. This implies that a rise in plasma magnesium will inhibit secretion of parathyroid hormone producing an increased excretion of magnesium in the urine and a return of the plasma level towards normal. A fall in plasma magnesium will stimulate production of parathyroid hormone, leading to increased renal conservation. The hypothesis also implies that calcium and magnesium homœostasis are interdependent and that calcium homœostasis will not be maintained in the face of marked abnormalities in plasma magnesium.

However, there is a substantial amount of evidence in favour of this view. First, experimental magnesium deficiency in the rat produces hypercalcæmia, hypophosphatæmia and increased phosphate clearance[2], suggesting over-secretion of parathyroid hormone. Secondly, magnesium, administered by stomach tube, can prevent the marked increase in osteoclasts usually found in the femur of nephrectomized rats[3]. This suggests that elevation of plasma magnesium inhibits secretion of parathyroid hormone. Thirdly, total parathyroidectomy in the rat is followed by a fall in plasma magnesium, although the fall is not as marked as that seen in calcium[4].

The effect of parathyroid hormone on urine calcium must also be of homœostatic importance, although overshadowed by the effect of the hormone on bone. In the case of magnesium, however, the renal effect may be more important, since the calcium : magnesium ratio in bone is 50 : 1, while the renal conserving effect of parathyroid hormone results in similar retentions of calcium and magnesium.

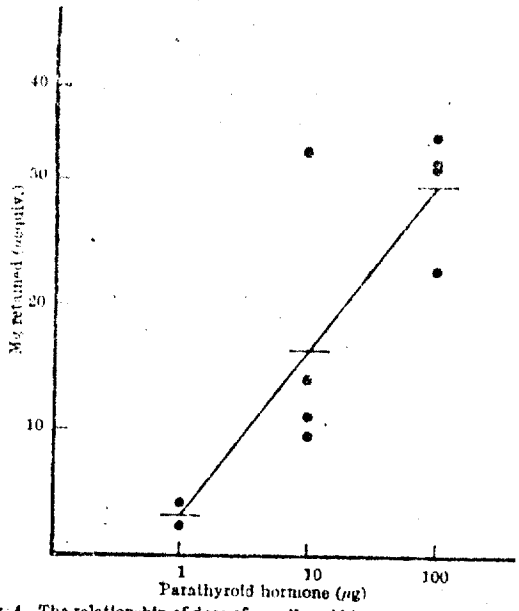

Fig. 4. The relationship of dose of parathyroid hormone and magnesium retained

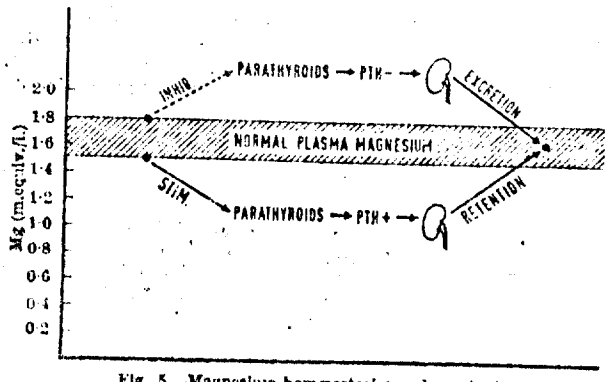

Fig. 5. Magnesium homœostasis: a hypothesis

We thank Dr. Howard Rasmussen for supplies of pure parathyroid hormone, Eli Lilly and Co. for supplies of parathyroid extract, and Technicon, Ltd., for loan of equipment. We are grateful to the Medical Research Council for financial support.

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MICROMETHOD FOR THE ESTIMATION OF MAGNESIUM IN BIOLOGIC FLUIDS

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SUMMARY

A micromethod for the magnesium in body fluids, based on the red magnesium hydroxide–Titan yellow complex, has been presented. The reagents have been stabilized and their preparation greatly facilitated. The interferences have been thoroughly studied and clearly elucidated. It is our opinion that, for the practical analysis of biologic fluids for clinical purposes, they have been largely exaggerated in the extant literature. The stoichiometry of the reaction has been carefully worked out. Reliability of the method has been established by comparison with two other methods, by recovery experiments and by replicate analyses. We believe it to be the fastest, simplest, and most meticulously established method for magnesium in biologic fluids available.

The literature is replete with references to the determination of magnesium in biologic fluids. A recent survey of members of the American Association of Clinical Chemists by RICE AND GROGAN[1] indicated that, among the 270 respondents, 139 performed magnesium determinations and these employed 25 different technics for determination of this parameter.

Four chemical approaches to the determination of biologic magnesium have been developed. One of the oldest depends upon the precipitation of magnesium as magnesium ammonium phosphate[2]. The method requires preliminary calcium removal. After purification and redissolution of the double salt, the phosphate is determined as inorganic phosphorus.

A second chemical method involves precipitation of magnesium as magnesium 8-hydroxyquinoline by the addition of 8-hydroxyquinoline at an alkaline pH in the method of HOFFMAN[3]. The resulting compound is then determined colorimetrically. SCHACTER[4] modified the technic by estimating the compound fluorometrically after dissolution in ethanolic solution. Recently, the procedure has been automated by HILL[5] by adapting the flow stream analysis system of the AutoAnalyzer*** to a recording fluorometer.

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and 2 ml of reagent-grade 37% formaldehyde (HCHO) to 200 ml with distilled water. This solution is stable at least 1 yr. at room temperature.

Stock 0.075% (w/v) Titan Yellow. In about 800 ml of distilled water dissolve 0.75 g of Titan yellow* (Clayton yellow). Add 10 ml of reagent-grade 37% formaldehyde (HCHO) and dilute to 1 l with distilled water. Filter through Whatman No. 42 filter paper. This solution is stable at least 1 yr. at room temperature.

0.075% (w/v) Titan Yellow. Dilute 2 ml of stock 0.075% (w/v) Titan yellow to 10 ml with distilled water. This solution is stable at least 12 days at room temperature and at least 1 month in the refrigerator (approx. 5°).

10% (w/v) Sodium Hydroxide. Dissolve 25 g of reagent-grade sodium hydroxide

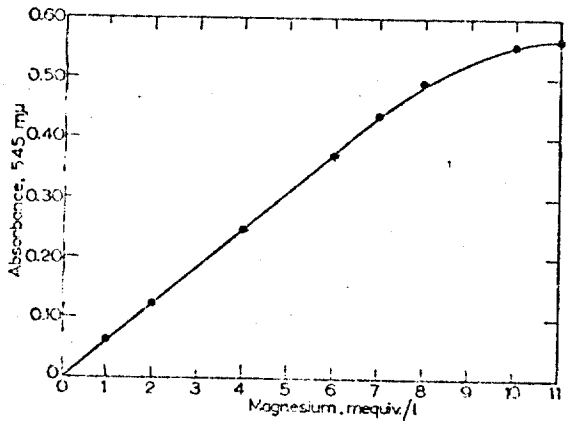

Fig. 1. Typical calibration curve obtained on a Coleman Junior Spectrophotometer, Model 6D.

(NaOH) pellets in and dilute to 250 ml with distilled water. Store in a polyethylene bottle. This solution is stable if kept tightly stoppered.

Calibration procedure

In each of eight 100-ml volumetric flasks place 1.0, 2.0, 4.0, 6.0, 7.0, 8.0, 10.0 and 11.0 ml of magnesium stock standard, 100 mequiv/l. Dilute to the 100-ml mark in each case with distilled water. These solutions are equivalent to 1, 2, 4, 6, 7, 8, 10 and 11 mequiv/l of magnesium. Use 0.2 ml of each solution in place of the working standard in the routine procedure. Prepare a graph relating mequiv/l of magnesium to absorbance. This curve shows sufficient daily variability to necessitate using a concurrent standard in the routine procedure. However, the curve should be drawn to determine the extent of adherence to the Beer-Lambert law with various photoelectric instruments. A typical calibration curve is shown (Fig. 1).

Assay procedure

To 0.4 ml of distilled water in a test tube (13 × 100 mm) add 0.2 ml of serum, urine, sweat or cerebrospinal fluid. Add 0.3 ml of 15% (w/v) trichloroacetic acid. Mix well by lateral shaking. Centrifuge at moderate speed for 2–3 min. Pipet 0.5 ml of supernatant fluid and 1.0 ml of 0.05% (w/v) polyvinyl alcohol into a round cuvet

* Cat. No. 1835 Hartman-Leddon Co., Philadelphia, Pa. (U.S.A.) *or* Cat. No. 4454 Eastman Kodak Co., Rochester, N.Y. (U.S.A.).

and mix. Add 0.5 ml of 0.015% (w/v) Titan yellow, mix, and then add 1.0 ml of 10% (w/v) sodium hydroxide. Mix by lateral shaking. Within the next 10 to 25 min, read the optical density at 545 mμ against a reagent blank prepared by treating 0.2 ml of distilled water exactly as the unknown. The working standard is also treated exactly as the unknown. The color is linear up to 7 mequiv magnesium/l. In the case of urine, subtract 0.2 mequiv/l from the calculated value to correct for the average contribution of urine pigments to the final results. .

All the figures quoted in this paper were obtained with a Coleman Junior Spectrophotometer, Model 6D* using 19-mm round cuvets. To permit readings on as little as 3 ml of solution in the 19-mm cuvets, a slice of rubber was cut from a solid rubber stopper of sufficient thickness to elevate 3 ml of solution, contained in the cuvet, into the optical path when the rubber slice was placed in the bottom of the cuvet adapter.

RESULTS

Spectral characteristics of the reaction mixture

Absorption spectra were determined on pooled urine, pooled cerebrospinal fluid, pooled serum and on a standard (2 mequiv/l). Fig. 2 graphically depicts the results of this work. Maximum absorption occurred at 545 mμ.

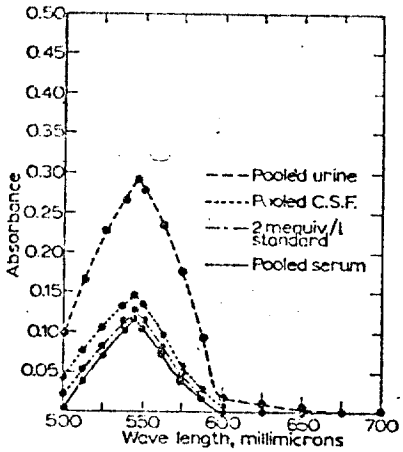

Fig. 2. Absorption spectra of modified Titan yellow method in various biologic fluids.

Stability of final colored lake

Fig. 3 shows the stability of the final color when the Titan yellow reaction is conducted with various magnesium-containing fluids. A useful plateau of color stability occurs between 10 and 25 min. Data beyond 60 min are not shown in Fig. 3 to permit greater clarity and detail.

Optimal concentration of Titan Yellow solution

The Titan yellow solution was varied in strength between 0.0075% (w/v) and 0.015% (w/v). As the concentration increased, the blank became increasingly more

* Coleman Instruments, Inc., Maywood, Ill. (U.S.A.).

intense so that at a concentration of 0.17% (w/v), it became impossible to set the instrument to optical zero with the blank, thus necessitating reading both the blank and sample against water. However, as the concentration of dye increased, linearity of the final color increased from 5 mequiv magnesium/l to 7 mequiv/l at the level of 0.015% (w/v) used in the present method. However, there was no difference in measured color intensity within the range of coincident linearity. We chose the latter concentration as a compromise between color in the blank, linearity and methodologic simplicity.

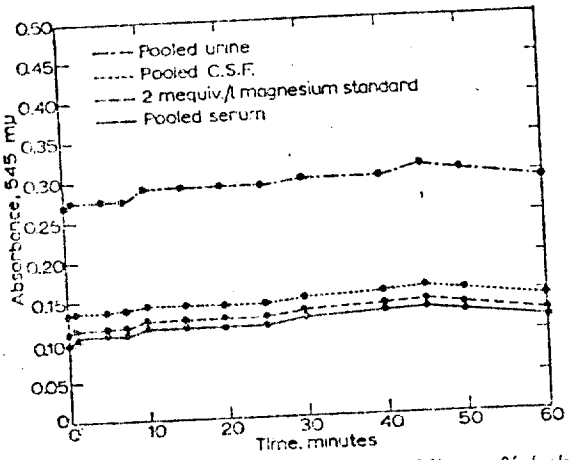

Fig. 3. Change in absorbance of final color with time after adding 10% (w/v) sodium hydroxide.

The stoichiometry is further illustrated by using the dye exactly as in the present method but doubling the amount of sample (proportionately reducing the amount of water used). This resulted in exactly twice the color intensity but strict adherence to the Beer-Lambert law prevailed only to about 2.5 mequiv magnesium/l.

Strength of alkali used

No difference in results was obtained when the alkali was varied from 8% (w/v) to 15% (w/v). 10% (w/v) sodium hydroxide was chosen as a compromise, permitting easy preparation of the solution and yet allowing considerable error in its preparation.

Choice of dispersing colloid

Various dispersing and stabilizing agents have been tried with Titan yellow for the estimation of biologic magnesium. The agents used included starch[14], gelatin[15], hydroxylamine hydrochloride[16], gum ghatti[7] and polyvinyl alcohol[8]. For various reasons, we limited the final choice to one between gum ghatti and polyvinyl alcohol. A 0.05% (w/v) gum ghatti solution was prepared in the same fashion as the 0.05% (w/v) polyvinyl alcohol solution used routinely. The gum ghatti solution was used in the routine procedure in exactly the same manner as the polyvinyl alcohol. Peak absorption, when gum ghatti is used, occurs at 550 mμ as opposed to 545 mμ in the routine assay using polyvinyl alcohol. Comparative results with the two dispersing colloids are shown in Table I. The final color using each colloid was read at the respective peak absorption previously observed. Despite the somewhat higher blanks

with polyvinyl alcohol, it is superior to gum ghatti in affording greater sensitivity and somewhat greater color stability. Furthermore, the polyvinyl alcohol solution is considerably easier to prepare. SPARE[17] got results with gum acacia that were identical to those with gum ghatti. He also found polyvinyl alcohol superior, and found dextrans and human albumin of no value for increasing color intensity. SPARE[17] did find that human γ-globulins intensified the color to the same degree as polyvinyl alcohol.

Choice of deproteinizing agent

Stabilized tungstic acid, as formulated by KRAUTMAN[18], may be used as a direct replacement of 15% (w/v) trichloroacetic acid in the routine assay. However, this results in a drop of about 7% in color density. Thus, despite a slightly higher blank, it is preferable to use trichloroacetic acid as the deproteinizing agent.

TABLE I

COMPARISON OF TWO DISPERSING COLLOIDS

Specimen	*0.05% (w/v) Gum Ghatti (Optical density)*	*0.05% (w/v) Polyvinyl Alcohol (Optical density)*
Serum	0.088	0.106
2 mequiv/l Standard	0.104	0.125
4 mequiv/l Standard	0.207	0.251

We would not advocate eliminating the deproteinizing agent completely in this method as suggested by SPARE[17]. By doing so, we obtained results that averaged about 7% higher than with deproteinized serum and they were somewhat erratic. We do agree, however, with SPARE's observation[17] that serum should be diluted prior to assay or low results and poor recoveries ensue.

Effects of potential interfering agents

Calcium. Addition of as much as 15 mequiv/l of calcium to the working standard produced no change in final absorbancy. This corroborates the work of SPARE[17] and extends it.

Gluconate. ANAST[20] found that a Titan yellow method, modified from that of ORANGE AND RHEIN[19], gave false low results in the serum and urine of patients receiving intravenous calcium gluconate. We confirmed this finding.

Mercury. Recently, BARKER *et al.*[21] found that mercurial diuretics resulted in falsely low urine magnesium levels in patients receiving these drugs. We confirmed this effect.

Phosphate and ammonium ions. Amounts equivalent to twice the upper physiological limits for these ions produced no effect on serum analyses with the present method.

Use of anticoagulants

Potassium oxalate, ammonium heparin, sodium citrate or the disodium salt of ethylenediamine tetraacetic acid may be used as anticoagulants with no change in values stemming from analysis of the resulting plasma.

Effect of hemolysis

The effect of hemolysis occurs in two stages. With moderate hemolysis, the values are slightly elevated due to the higher concentration of magnesium in red cells as opposed to serum. However, when the hemolysis becomes massive, the values become abnormally low. The reduced values apparently are the net effect of hemoglobin iron and enrichment from intracellular magnesium, with the iron effect predominating. The diminution in values is proportional to the concentration of extravasated iron. This can be demonstrated by adding ferric iron to serum samples. The effect, however, cannot be demonstrated on protein-free filtrates. The effect may be due to a change produced in the protein-bound fraction of magnesium which causes the magnesium to be more tightly bound to protein and not released by the deproteinizing agent. Thus, only very *slight* hemolysis can be tolerated. ORANGE AND RHEIN[19] used cupferron for removing non-physiologic levels of iron. This should not pose as a practical problem.

Effect of lipemia

We could demonstrate no effect from lipemia in serum on the present method. Therefore, fasting blood samples are not mandatory.

Effect of bilirubinemia

Values of serum total bilirubin up to 32 mg/100 ml were found to have no effect on the determination of serum magnesium by the present method. If the serum is not deproteinized, however, each 1 mg of bilirubin/100 ml raises the serum magnesium level by about 0.05 mequiv/l.

Precision

Thirty serums were analyzed in duplicate. The standard deviation, calculated by the formula[13]

$$\text{S.D.} = \sqrt{\frac{\Sigma (x_1 - x_2)^2}{N - 1}}$$

was 0.04 for the present method. Since there is usually no need for deproteinization in urine, sweat or cerebrospinal fluid, the precision would be expected to be at least as good as for serum. Normal levels of sweat magnesium are measured at suboptimal regions of instrumental sensitivity and the precision might be expected to suffer accordingly.

Recovery of added magnesium

Recovery of magnesium was studied by adding known concentrations of magnesium to serum. Results are shown in Table II. The excellent recoveries are consistent with the good precision found.

Comparison of methods

In order to validate this micro method, the present method was compared with the macro Titan yellow method of HEAGY[8] and the ammonium phosphate method of BRIGGS[2] relative to serum analyses. The results are given in full in Table III, from which it may be seen that, in general, the agreement was excellent between the two

Titan yellow methods, but that the ammonium phosphate method of BRIGGS[2] gave results, on an average, 5% higher than the Titan yellow methods.

Stability of magnesium on body fluids

Serum should be separated from the clot within ½ h of collection but the analysis may be delayed at least 24 h at room temperature (approx. 25°). Analyses may be deferred indefinitely if serum, urine, sweat and cerebrospinal fluid are kept frozen (approx. −15°).

TABLE II

RECOVERY OF MAGNESIUM ADDED TO SERUM

Magnesium added mequiv/l	*Magnesium found mequiv/l*	*% Recovery*
0.0	1.54 ± 0.04 (10)*	—
0.50	2.03 ± 0.04 (8)	97.0– 99.1
1.00	2.52 ± 0.04 (9)	97.1– 98.7
2.00	3.54 ± 0.03 (11)	99.3–100.8

* Mean ± S.D. Figures in parentheses are number of samples studied, in duplicate.

TABLE III

COMPARISON OF DIFFERENT SERUM MAGNESIUM METHODS

Specimen No.	*Serum magnesium mequiv/l according to*		
	this paper	*method*[4]	*method*[2]
1	1.76	1.78	1.84
2	1.87	1.84	1.96
3	1.76	1.79	1.83
4	1.64	1.66	1.74
5	1.84	1.83	1.94
6	1.80	1.79	1.93
7	1.91	1.92	2.02
8	2.06	2.06	2.14
9	1.79	1.83	1.90
10	1.55	1.59	1.64
11	1.73	1.70	1.82
12	1.99	1.95	2.11
13	1.72	1.74	1.81
14	1.68	1.64	1.76
15	1.83	1.82	1.92
Mean	1.80	1.80	1.89

Normal values

Thirty five fasting serum samples and twenty 24-h urine specimens, preserved with toluene, were obtained from hospital personnel having no signs of illness. Both sexes were about equally represented in the groups used for serum and urine collections and ranged in age from about 18 to 60 years. Sixteen specimens of sweat were obtained from pediatric patients and eighteen specimens of cerebrospinal fluid were obtained from adult general hospital patients with no clinical history of magnesium deficiency or excess. Magnesium determinations were performed on the serums within 1 h after collection of the blood at room temperature (approx. 25°), on the urine samples within 4 h after collection and refrigeration at 5°, and on the sweat and cerebrospinal fluid samples within 1 h after collection and refrigeration at 5°.

The 95% limits for serum magnesium were 1.4–2.4 mequiv/l and for urine magnesium were 1.5–9.5 mequiv/24 h.

The 95% "normal" limits for sweat magnesium were 0.1–0.5 mequiv/l and for cerebrospinal fluid were 0.9–3.1 mequiv/l.

No statistically significant difference between male and female levels was found in any of the body fluids.

DISCUSSION

We obtained extremely good results with the use of formaldehyde in preserving both the polyvinyl alcohol and Titan yellow solutions. We consider this a distinct contribution to the simplification of the Titan yellow method. CARAWAY (personal communication) has obtained fairly good stability by dissolving the Titan yellow in methyl cellosolve. However, color development is slower in this medium and color stability is not as good as in an aqueous medium. Sodium azide proved a good preservative but interfered with the color reaction. It should be pointed out that additional formaldehyde added to the 0.015% Titan yellow solution was not found effective.

The 0.05% (w/v) polyvinyl alcohol and 0.015% (w/v) Titan yellow may be premixed in the ratio of 2:1. This mixture is stable for only one week at room temperature or in the refrigerator. Unless the work load is very heavy, we do not recommend this expedient. We strongly caution against adding the 10% (w/v) sodium hydroxide to this mixture for any protracted storage period. Polyvinyl alcohol shows poor resistance to both strong acids and alkalis, and should not be exposed to the sodium hydroxide for long periods.

The wavelength used, 545 mμ, is critical as seen in Fig. 2, and is valid for the Coleman Junior Spectrophotometer. It is well to check the wavelength calibration of this instrument quite often with a didymium filter when performing magnesium assay. In this way, maximum sensitivity can be assured. If other instruments are used, check the 540–545 mμ region for maximum absorbance. Filter photocolorimeters may give capricious results. The use of a concurrent standard somewhat minimizes these considerations.

Turbidity in the trichloroacetic acid protein-free centrifugate, which sometimes occurs, disappears on the addition of alkali and apparently does not interfere with the determination. It has been stated that filtration avoids this turbidity. However, the danger of picking up adventitious magnesium from the filter paper must be kept in mind. Further, filtration does not subserve a micro technic.

With the use of a concurrent standard, the variation in commercial lots of Titan yellow is not as important and we do not specify that the dye be purified before use. We found no difference in actual use in the following commercial Titan yellow powders: (1) Eastman #P4454 (Eastman Kodak Co., Rochester, N.Y. (U.S.A.)), (2) Hartman-Leddon #1835 (Hartman-Leddon Co., Philadelphia, Pa. (U.S.A.)), and (3) National Aniline Indicator Cat. No. 228 (National Aniline Division, Allied Chemical [illegible] New York, N.Y. (U.S.A.)).

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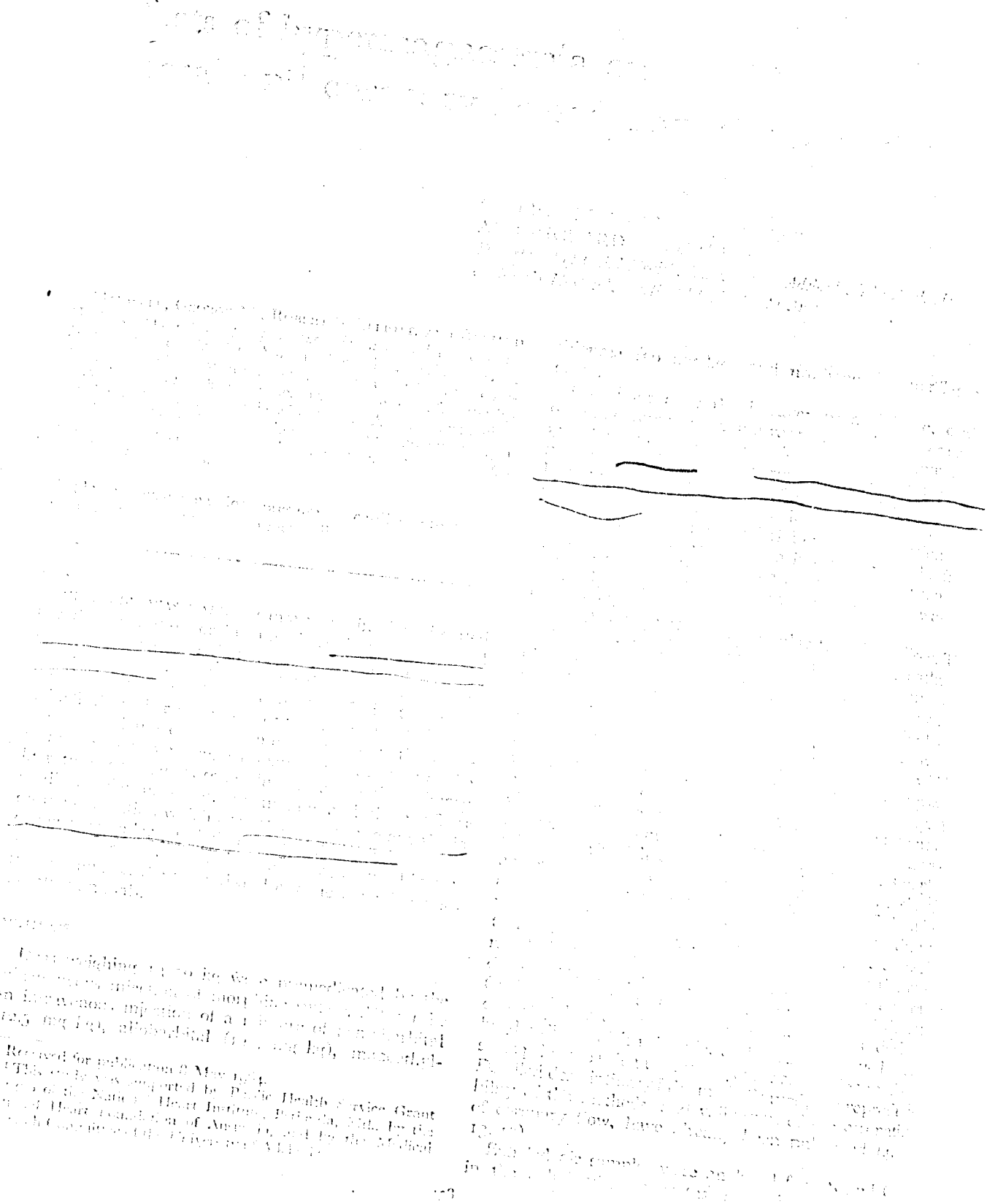

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TABLE 1. *General and coronary hemodynamics and [illegible]*

The following not noted in Table 1 were significantly increased: sinus coronary flow, 0.2 ± 0.3 to 0.7 ± 0.3 ml/100 g heart per min, and stroke cardiac oxygen consumption (0.1 ± 0.02 to 0.07 ± 0.03 ml), derived in this instance by division of the cardiac oxygen consumption per minute by the mean pulse rate during the time of estimation of the coronary flow.

Discussion

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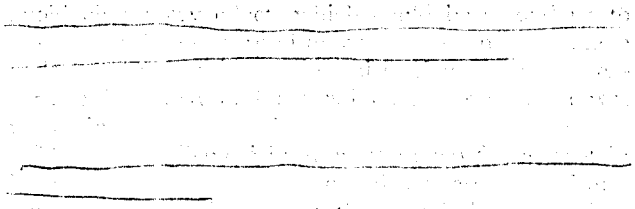

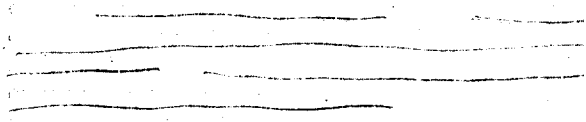

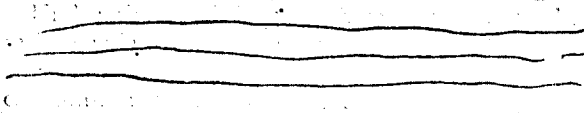

Mineral Balance Studies with the Baby Pig: Effects of Dietary Magnesium Level upon Calcium, Phosphorus and Magnesium Balance [1,2]

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ABSTRACT Calcium, phosphorus and magnesium balance studies were conducted with baby pigs receiving a purified casein-glucose diet containing levels of magnesium of 75, 225, 325, 425 and 825 ppm. Dietary levels of Ca and P were maintained at 0.8 and 0.6%, respectively, with a constant dietary vitamin D_3 level of 1800 IU/kg. Food intake and consequent mineral intake were reduced in animals receiving 75 ppm dietary Mg. Increasing dietary Mg level above 225 ppm did not significantly affect Ca or P balance but the maximal percentage retention of Ca and P was obtained with pigs receiving 325 ppm of dietary Mg. Urinary Ca excretion was significantly increased by increased dietary Mg. Excreta Mg and Mg balance were both positively related to Mg intake. Maximal percentage Mg retention was obtained with dietary Mg levels of 225 or 325 ppm.

Studies by Hart and Steenbock (1) with adolescent pigs have shown that the addition of magnesium salts to a basal bran diet results in increased urinary calcium elimination and may result in a negative calcium balance. Studies by Bartley et al. (2), Freese (3) and Lenkeit and Freese (4) with baby pigs receiving a purified diet or milk diet have shown that magnesium balance is positively related to magnesium intake. Studies with other laboratory animals have generally shown adverse effects of increased dietary calcium and phosphorus upon dietary magnesium utilization (5), but increasing dietary magnesium has not generally greatly affected calcium and phosphorus utilization (6).

The present study was undertaken to determine the effects of dietary magnesium upon calcium, phosphorus and magnesium utilization by the baby pig as determined by mineral balance trials and to provide supplementary information toward a more accurate determination of the magnesium requirement of the baby pig (7).

MATERIALS AND METHODS

Baby pigs used in this study were from the second of 2 trials conducted to determine their magnesium requirement (7) when fed purified casein-glucose diets. Calcium, phosphorus and magnesium balance studies were conducted with pigs receiving 75, 225, 325, 425 or 825 ppm. Dietary levels of Ca and P were 0.8 and 0.6%, respectively. Dietary sources of Ca, P and Mg were identical with those used in previous balance studies (8–10). Vitamin D_3 was supplied by cod liver oil[3] at a dietary concentration of 1800 IU/kg.

Twenty-nine mineral balance determinations were made when the baby pigs were 4 to 6 weeks of age. Methods of performing the balance trials were similar to those described previously (10). Three 3-day collections with controlled intakes were made on 2 animals receiving dietary Mg at each level with the exception that the final collection for one of the pigs receiving 75 ppm of dietary Mg was not completed because it died suddenly in Mg-deficiency tetany. A 3-day adjustment period with identical intake preceded each collection period. Distilled water served as the sole source of drinking water. Analyses of food, fecal and urine Ca, P and Mg were performed by methods indicated in a previous

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[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal article no. 3473.

[2] Presented in part before the meeting of the American Institute of Nutrition, April, 1963 (Federation Proc., 22: 491, 1963, abstract).

[3] Standardized Cod Liver Oil (6.25 μg vitamin D_3/g), Parke, Davis and Company, Detroit.

study (10). Data were examined by analysis of variance, and statistical significance of treatment differences was determined by the multiple range test of Duncan (11). Correlation coefficients were determined for certain factors of mineral balance and tested for significance (12).

RESULTS AND DISCUSSION

The summarized data from the balance studies are presented in table 1. Both of the pigs receiving 75 ppm of dietary Mg had depressed appetites which necessitated reduced controlled food intakes and resulted in reduced mineral intakes. There was no depressing effect upon food intake of pigs receiving higher dietary Mg concentration, and identical near-maximal controlled intakes were maintained in simultaneous collections. Increasing the dietary Mg concentration above 225 ppm did not significantly affect fecal Ca or P, urine P, Ca or P balance, or Ca or P retention. Maximal Ca or P balance and the

TABLE 1

Daily calcium, phosphorus and magnesium excretion and retention as affected by dietary magnesium level

	Dietary Mg, ppm[1]				
	75	225	325	425	825
No. of collections	5	6	6	6	6
Daily food intake, g	300 ± 33[a]	433 ± 48[b]	433 ± 48[b]	433 ± 48[b]	432 ± 48[b]
Ca balance					
Daily Ca intake, g	2.40 ± 0.26	3.46 ± 0.38[b]	3.46 ± 0.38[b]	3.46 ± 0.38[b]	3.46 ± 0.26[b]
Daily fecal Ca, g	0.46 ± 0.08	0.43 ± 0.05[a]	0.33 ± 0.06	0.47 ± 0.10	0.43 ± 0.10
Daily urinary Ca, g	0.002 ± 0.001	0.006 ± 0.001	0.014 ± 0.004	0.011 ± 0.001	0.011 ± 0.003[d]
Daily Ca retention, g	1.94 ± 0.24	3.03 ± 0.35[b]	3.10 ± 0.34[b]	2.98 ± 0.38[b]	2.99 ± 0.33[b]
Ca retention, %	81 ± 3	87 ± 1	89 ± 2[b]	86 ± 2	86 ± 2
P balance					
Daily P intake, g	1.80 ± 0.20	2.60 ± 0.29[b]	2.60 ± 0.29[b]	2.60 ± 0.29[b]	2.60 ± 0.29[b]
Daily fecal P, g	0.22 ± 0.04	0.22 ± 0.03	0.20 ± 0.01	0.27 ± 0.05	0.28 ± 0.06
Daily urinary P, g	0.22 ± 0.05	0.32 ± 0.06	0.29 ± 0.04	0.33 ± 0.08	0.33 ± 0.07
Daily P retention, g	1.36 ± 0.14	2.06 ± 0.25[b]	2.11 ± 0.24[b]	2.00 ± 0.18[b]	1.99 ± 0.20[b]
P retention, %	76 ± 3	79 ± 2	81 ± 2	76 ± 3	77 ± 3
Mg balance					
Daily Mg intake, mg	23 ± 2	97 ± 13[b]	141 ± 18[b,c]	186 ± 21[b,c,d]	357 ± 39[d]
Daily fecal Mg, mg	8 ± 1	21 ± 3[b]	25 ± 3	39 ± 5[b,c]	145 ± 24[b,d]
Daily urinary Mg, mg	0.4 ± 0.1	1.2 ± 0.4	1.4 ± 1	9 ± 1[b,c,d]	20 ± 3[d]
Daily Mg retention, mg	14 ± 2	75 ± 9[b]	100 ± 14[b]	126 ± 14[b]	192 ± 38[b,c,d]
Mg retention, %	62 ± 3	77 ± 2[a,b]	76 ± 5[a,b]	68 ± 4[a]	52 ± 6

[1] Mean ± SE.
[a] Significantly greater than least value ($P < 0.05$); ** $P < 0.01$.
[b] Significantly greater than least two values ($P < 0.05$); ** $P < 0.01$.
[c] Significantly greater than least three values ($P < 0.05$); ** $P < 0.01$.
[d] Significantly greater than all other values ($P < 0.05$); ** $P < 0.01$.

percentage Ca or P retention were obtained with pigs receiving 325 ppm of dietary Mg. Urine Ca excretion was significantly increased by increased dietary Mg intake with a correlation coefficient of 0.73 ($P < 0.01$). Serum Ca and Mg concentrations in these animals were positively related as were also serum Mg concentration and Mg intake (7). The increased serum Ca concentration present concomitantly with increased serum Mg concentration as a consequence of increased Mg intake could account for the increased urinary Ca without changing the secretory constant for renal clearance (13). The failure of dietary Mg level to affect Ca or P balance other than urinary Ca is illustrated in figures 1 and 2.

The data in table 1 indicate that increasing dietary Mg concentration results in both increased excreta Mg and increased Mg retention. This is in agreement with previous observations (2, 3) and is well illustrated in figure 3. This is further verified by the significant relationship between the factors of Mg intake, excretion and retention. The correlation coefficients of all animals for these factors were: Mg intake and Mg retention (0.91), Mg intake and excreta Mg (0.90), Mg intake and

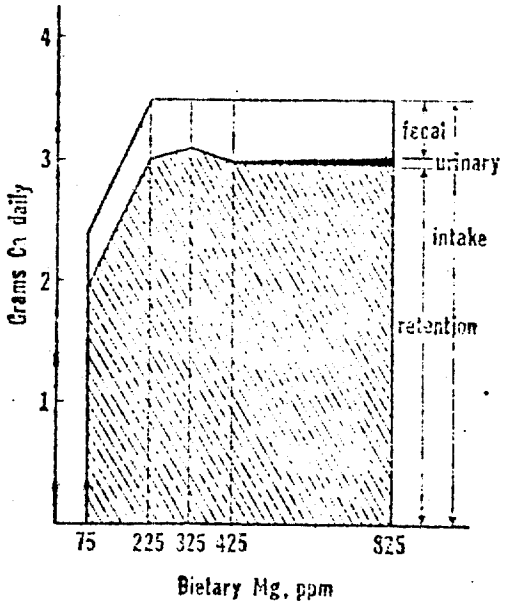

Fig. 1 Calcium balance as affected by dietary Mg level.

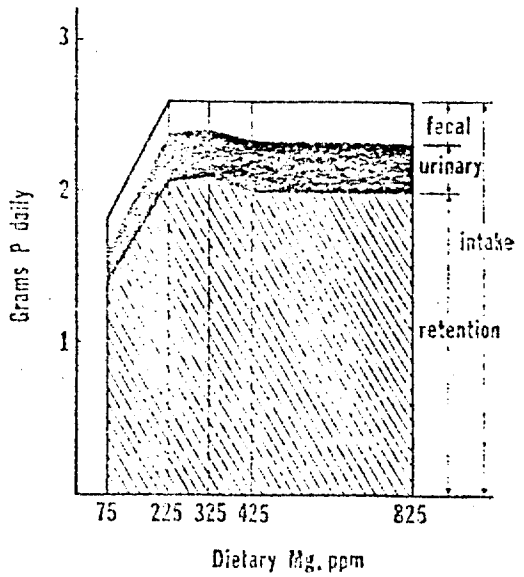

Fig. 2 Phosphorus balance as affected by dietary Mg level.

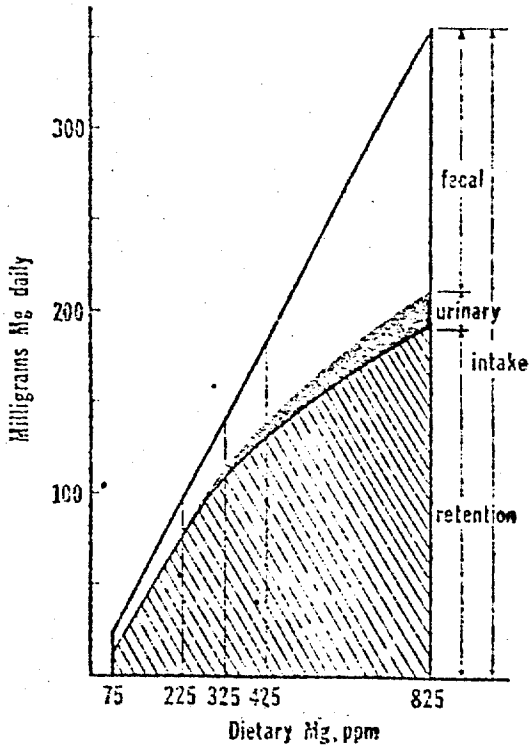

Fig. 3 Magnesium balance as affected by dietary Mg level.

fecal Mg (0.87), fecal Mg and urinary Mg (0.79), Mg intake and urinary Mg (0.75) and Mg retention and excreta Mg (0.61). All of these correlation coefficients were statistically significant ($P < 0.01$). When Mg intake and the percentage Mg retention of all animals were correlated the coefficient was -0.32 (nonsignificant); however, when values for the Mg-deficient pigs (75 ppm) were not considered in this correlation the coefficient became -0.53 and statistically significant ($P < 0.01$).

Pigs in this study (excluding those receiving dietary Mg at the lowest level) made an average body weight gain of 300 g daily during the course of the balance trials. Using the assumption of Bartley (2) that Mg concentration of the pig's body is 0.03%, the required daily Mg retention to maintain this body Mg concentration is 90 mg. Pigs receiving 325 ppm of dietary Mg retained 109 mg daily which appears to be an adequate margin of safety.

Data obtained in this study corroborate the work of Forbes (6) with the rat, in that increasing the level of dietary Mg did not significantly affect daily Ca or P retention but increased daily Mg retention. The balance data do not indicate a higher dietary Mg requirement than that of 325 ppm determined by other criteria (7).

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VOL. 1 (1956) CLINICA CHIMICA ACTA ; 577-582

SPECTROCHEMICAL DETERMINATION OF MAGNESIUM, CHROMIUM, NICKEL, COPPER AND ZINC IN HUMAN PLASMA

by

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The purpose of this investigation was to develop a spectrochemical procedure for the determination of the following elements in human plasma: magnesium, chromium, nickel, copper and zinc. It is part of a long-range study of the trace element content of human blood being conducted in co-operation with the Hematology Laboratory of the University of Virginia School of Medicine.

APPARATUS

Spectrograph. Applied Research Laboratories 2-meter grating spectrograph, modified with exterior optics and ignitor [1].

Excitation source. Applied Research Laboratories rectifier unit, 0–15 amperes direct current.

Densitometer. Applied Research Laboratories film projection comparator-densitometer.

Calculating equipment. A calculating board was employed to convert densitometer readings to intensity ratios by means of the film calibration curve.

REAGENTS

Hydrochloric acid. Baker and Adamson. Reagent grade. Qualitative spectrographic analysis showed the acid to be free of the elements to be determined.

Nitric acid. Baker and Adamson. Reagent grade. The acid was distilled in an all-Pyrex distilling apparatus.

Hydrogen peroxide, 30%. Fisher certified reagent.

Sodium heparin, solid salt. USP, Organon Inc., Orange, N.J.. Qualitative spectrochemical analysis of a sample showed it to be free of the metals to be determined.

Triply distilled water. Laboratory distilled water was passed through an exchange resin column (Dowex-50 × 12; 50–100 mesh) and then distilled twice more from two glass stills in series and automatically controlled. The second still was fitted with a fused silica condenser tube and the water was collected and stored in polyethylene bottles.

EXPERIMENTAL

A. Cleaning procedure

All apparatus coming in contact with the plasma samples should be cleaned in the following manner:

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(1) Wash well with detergent and water using a test tube brush for scrubbing.

(2) Soak in a cleaning solution for at least half an hour. The cleaning solution is a mixture of nitric and sulfuric acid; better to use hot nitric acid.

(3) Rinse with tap water; then rinse six times with distilled water.

(4) Rinse with triply distilled water.

B. Preparation of the sample

It is necessary to draw the blood from the donor in a very careful manner in order to avoid contamination. The sample is drawn as described by THIERS, WILLIAMS AND YOE [2] in their work on cobalt, using a 50 milliliter hypodermic syringe, fitted with a special platinum-ruthenium alloy needle (J. Bishop and Co., Platinum Works, Malvern, Pa.). Before using, the needle is cleaned in the same manner as in washing glassware, with the exception that it is immersed in cleaning solution for only 20 or 30 seconds. The clean syringe and needle are then placed in glass jackets and sterilized by heating them in an oven at 180° C overnight. One and one-half milligrams of sodium heparin are added to the chamber of the syringe in order to prevent coagulation of the sample during drawing. The plasma is obtained by centrifugation in a polyethylene bottle and the sample is kept under refrigeration.

C. Digestion of the sample

Many reagents for wet ashing are available. Their advantages and disadvantages are well demonstrated in an extensive study by MIDDLETON AND STUCKEY [3] and by SMITH and co-workers [4]. We found that the use of nitric acid and 30% hydrogen peroxide gave us the best reproducible results. The procedure for digesting plasma, which can be applied also to red cells and whole blood, is as follows:

(1) Take from the polyethylene bottle used for storage between 8 and 10 grams of plasma and pour into a 50 ml Erlenmeyer flask (3 to 3.5 g for red cells and whole blood).

(2) Add 2 ml of nitric acid, previously distilled in order to eliminate possible contamination, and 0.5 ml of hydrogen peroxide and heat on a hot-plate to slightly below the boiling point of the solution. When the material has reached a syrupy stage, the evolution of heavy brown vapor starts and the temperature is lowered in order to avoid a sudden "self-ignition". In fact, as MIDDLETON AND STUCKEY [3] pointed out, the temperature obtained locally for a short period is obviously quite high as indicated by bright "hot-spots" and it is to be expected that such a vigorous reaction might lead to loss of some metals. After this reaction subsides the black residue is left on the hot-plate, the temperature is raised, and the residue is brought to complete dryness.

(3) Place the flask in a mufile at 350° C overnight.

(4) Allow the flask to cool and add 1 ml of nitric acid. Put the mixture on the hot-plate and let it boil slowly to dryness. This operation is repeated once more.

(5) After cooling, 1 ml of hydrochloric acid is added to the mixture and again boiled to dryness.

(6) Add the internal standard to the residue and make up to 5 ml in a volumetric flask.

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TABLE I

VALUES FOR MAGNESIUM, CHROMIUM, NICKEL, COPPER AND ZINC IN HUMAN PLASMA

Element	*Content, γ/g*	*Range, γ/g*	*Method*	*Investigator*
Magnesium	24	10–42	Spectrochemical	MONACELLI, TANAKA AND YOE
	22.7	19–25	Colorimetric	ORANGE [5]
		32.3–37.3	Chromatographic	SEILER [6]
		17–29		HALD *et al.*[7]
Chromium	0.18	0.08–0.3	Spectrochemical	MONACELLI, TANAKA AND YOE
	0.02	0.007–0.5	Spectrochemical	KOCH *et al.*[8]
Nickel	0.04	0.01–0.06	Spectrochemical	MONACELLI, TANAKA AND YOE
	0.03	0.01–0.085	Spectrochemical	KOCH *et al.*[8]
	0.012		Colorimetric	CLUETT [9]
Copper	1.2	1.0–2.1	Spectrochemical	MONACELLI, TANAKA AND YOE
	0.77 ± 0.35			DAUM [10]
	1.1	0.87–1.61	Colorimetric	CARTWRIGHT [11]
	0.92 ± 0.19	0.67–1.3	Spectrochemical	KOCH *et al.*[8]
	0.98 ± 0.12	0.65–1.35	Colorimetric	*Ibid.*
Zinc	1.3	0.6–2.3	Spectrochemical	MONACELLI, TANAKA AND YOE
	1.13	0.72–1.6	Colorimetric	BERFENSTAM [12]
	2.4 ± 0.45		Colorimetric	HOCH [13]
		1.40–2.00	Colorimetric	WOLFF [14]
	M 3.90	1.20–11.40	Colorimetric	VALEE AND GIVSON [16]
	F 3.10	1.20–10.10	Colorimetric	*Ibid.*
	M 1.97	1.37–2.84	Colorimetric	WOLFF [17]
	F 1.94	1.41–2.72	Colorimetric	*Ibid.*
	M 1.28	0.84–1.57	Colorimetric	VIKBLADH [15]
	F 1.23	0.88–1.63	Colorimetric	*Ibid.*
	M 1.21	0.32–1.70	Colorimetric	KOCH *et al.*[8]
	F 1.19	0.79–1.50	Colorimetric	*Ibid.*

TABLE II

DATA ON CONTROL SAMPLES

Sample No.	*Element*	*Quantity added γ/g*	*Quantity recovered γ/g*
1	Magnesium	20	21
2	"	30	31.5
3	"	40	41.4
1	Chromium	0.2	0.18
2	"	0.3	0.33
3	"	0.4	0.38
1	Nickel	0.03	0.025
2	"	0.04	0.037
3	"	0.05	0.05
1	Copper	1.0	1.04
2	"	1.25	1.29
3	"	1.40	1.45
1	Zinc	1.0	0.92
2	"	1.3	1.25
3	"	1.5	1.46

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TABLE III

DATA ON PRECISION OF METHOD

Element	*Average concentration in γ/g*	*Coefficient of variation*	*Number of determinations*
Magnesium	24	1.60	25
Chromium	0.18	0.13	25
Nickel	0.04	0.45	12
Copper	1.2	1.16	22
Zinc	1.3	1.32	14

SUMMARY

Procedures are presented for the collection and digestion of human plasma for the spectrochemical determination of Mg, Cr, Ni, Cu and Zn. Data on the precision of the method are given. Values found for the five metals are compared with those given in the literature.

The procedure for digesting plasma can be applied also to red cells and whole blood.

RÉSUMÉ

Des méthodes sont proposées pour la préparation et le traitement de plasma humain, en vue d'un dosage spectrochimique des éléments suivants: Mg, Cr, Ni, Cu et Zn. La précision obtenue par ce procédé est donnée. Les résultats trouvés pour ces 5 métaux comparés avec ceux signalés dans la littérature. Le mode opératoire décrit pour la minéralisation du plasma peut être appliqué également aux globules rouges et au sang total.

ZUSAMMENFASSUNG

Verfahren werden vorgeschlagen, mittels derer menschliches Plasma, dessen Gehalt an Mg, Cr, Ni, Cu und Zn spektrochemisch bestimmt werden soll, gesammelt und vorbehandelt werden kann. Es werden auch Angaben über die Genauigkeit der Methode mitgeteilt. Die für die fünf Metalle gefundenen Werte werden mit denen der Literatur verglichen. Das Verfahren zur Vorbehandlung des Plasmas kann auch auf rote Blutkörperchen und auf Gesamtblut angewendet werden.

РЕЗЮМЕ

Предлагается метод сбора и настаивания человеческой плазмы для спектрохимического определения магния, хрома, никеля, меди и цинка. Приводятся данные о точности этого метода. Значения, найденные для этих пяти металлов сравниваются с имеющимися в литературе данными.

Описываемый процесс отстаивания плазмы может также быть применен к красным кровяным телам и ко всей крови.

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SCIENTIFIC NOTES

#2756

The Automated Fluorometric Determination of Serum Magnesium

Morris Oklander and Bernard Klein

A modified flow diagram and a simplified reagent have been developed for the automated fluorometric determination of serum magnesium by the Hill procedure (1).

THIS REPORT presents a modification of the automated fluorometric determination of serum magnesium described by Hill (*1*). Significant alterations in the manifold, reagents, and sampling procedure have been introduced. The sensitivity of the procedure has been increased by incorporating recent technical improvements.

Experimental

Reagents

Acetate buffer 1 *M*, pH 4.4, containing 2.0 mg./ml. potassium oxalate

Potassium chloride 4% (w/v)

Buffered alcoholic 8-hydroxyquinoline To a solution of 3.0 gm. tris-(hydroxymethyl)aminomethane, in 20 ml. distilled or deionized (preferred) water, 50 ml. absolute ethanol is added with magnetic mixing. To the clear solution, 0.35 gm. 8-hydroxyquinoline (Fisher Certified No. 0-261) is added with mixing. When dissolved the solution is diluted to 100 ml. with absolute ethanol. The solution should be stored in an amber polyethylene bottle.

Magnesium standards The standards are prepared from magnesium oxide as described by Schaeter (*2*) and cover the range from 0.5 to 4.0 mg. Mg^{++}/100 ml. (0.41–3.28 mEq./L).

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The capable clerical contributions of Mrs. Jean Meyer are gratefully acknowledged.

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Flow Diagram

The flow diagram is shown in Fig. 1. With the Sampler II,* specimens are sampled, diluted with buffered oxalate, dialyzed into potassium chloride solution, and mixed with the reagent. The resulting fluorescence is measured in a flow-cell fluorometer and recorded.

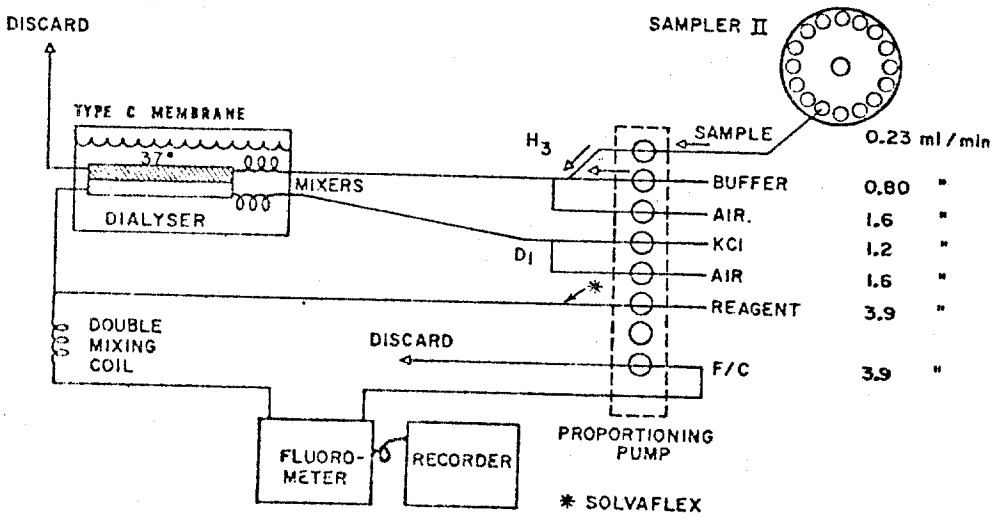

Fig. 1. Flow diagram for automated fluorometric serum magnesium determination.

Membrane material A Type C cupriphane dialysis membrane is used.

Fluorometer The Technicon fluorometer is used in this procedure. A Turner photofluorometer modified for automated operation can also be used.

Chart paper The chart paper used is ruled for per cent transmission; the chart speed is 18 in./hr.

Filters The primary (exciting) filter is a narrow-pass filter (Turner 110-812) with a peak at 405 mμ. It is a composite (Corning 7-51 and Wratten 2C). The secondary filter is a narrow-pass filter (Turner 110-822) with a peak at 525 mμ, color specification No. 58.

Aperture In this work a No. 4 aperture slit was used (see *Operating Procedure*) as was the 30× range setting. These conditions may be altered to accommodate local situations.

Operating Procedure

All solutions and reagents are aspirated for about 10 min. When the combined reagent stream passes through the flow cell, the fluorometer

*Technicon Instruments Corporation, Ardsley, N. Y.

baseline is adjusted with the blank knob to the 1% line on the chart paper. A 4.0 mg./100 ml. magnesium standard is continuously aspirated and the fluorescence is recorded. The fluorescence response should be between 60 and 80 (arbitrary units). If less than 60 or above 80, the slits are replaced to bring the response within this range. The baseline is rechecked and readjusted to the 1% line if necessary. The analyst is referred to the fluorometer operating instruction manual for details.

Standards and specimens are then aspirated at either the 30 specimen/hr. rate (2:1 wash ratio) or the 40 specimen/hr. rate (1:1 wash ratio). (The 40 specimen per hour (1:1) sampling cam is not usually supplied with the Sampler II but can be obtained from Technicon.)

Calibration Curve

Standard magnesium solutions containing 0.5–4.0 mg. Mg^{++}/100 ml. (0.41–3.28 mEq./L.) are analyzed and the fluorescence response, in arbitrary units, is plotted against concentration. It is recognized that magnesium concentrations should be expressed in milliequivalents per liter for meaningful clinical interpretation. This expression is readily obtained by multiplying milligrams Mg^{++} per 100 ml. by 0.82. A typical calibration curve and the strip chart recording from which it was constructed are shown in Fig. 2.

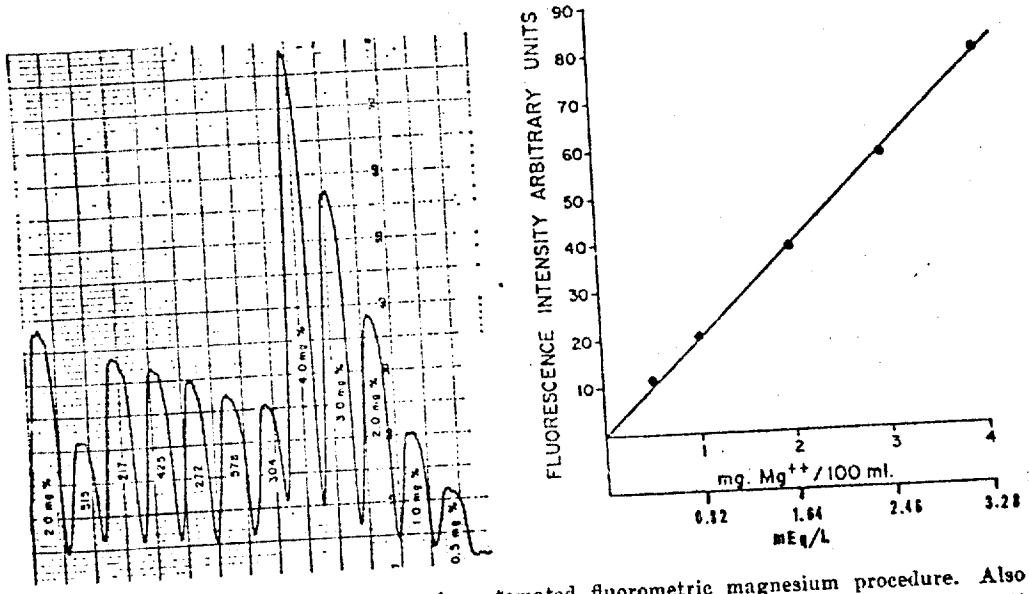

Fig. 2. Typical calibration curve in automated fluorometric magnesium procedure. Also shown are several serum analyses between two readings of 2 mg./100 ml. (1.64 mEq/L.) Mg^{++} standards. Aspiration was at the rate of 30/hr. (2:1 wash ratio).

Results

From Fig. 2, it is evident that the fluorescence response (arbitrary units) is linear to 4.0 mg. Mg^{++}/100 ml. The results of analyses of several serum specimens submitted to the clinical biochemistry laboratory for determination of magnesium content, obtained by the modified automated procedure, agreed within 3% with the values obtained by the manual fluorometric procedure (*2*), and determined on a Turner Model 111 Photofluorometer.

Recovery of magnesium added to serum and determined by the present method varied from 92.6 to 97%.

Discussion

During the development of the present procedure several modifications were introduced. The fluorometric reaction with the buffered alcoholic 8-hydroxyquinoline reagent was simplified by combining all the ingredients in a single solution. The single solution was added to the dialyzate by a single Solvaflex* manifold tube and mixed in one double mixing coil to insure complete reaction. No difference in response was noted when the Hill manifold and the modified manifold were compared. It was apparent that the new membrane material promoted better dialysis.

The modified reagent proved stable for at least 4 days at room temperature, although only 100-ml. quantities were prepared to insure fresh reagent. The reagent was checked daily by determinations of the deflection from the water baseline; it usually was between 20 and 25 units. The ability of the reagent to produce a fluorescence response between 60 and 80 units with the 4.0-mg. Mg^{++} standard served as an additional check on the reagent's stability.

Good sensitivity was obtained in the present procedure with almost half the sample size used by Hill: 0.24 ml./min. as compared with 0.42 ml./min. This distinct advantage is in part the result of improved dialysis with the Type C membrane. The decreased dilution of the dialysis stream—e.g., 3.9 ml. reagent per minute as compared with 6.0 ml./min. in the Hill procedure, also contributed to the increased fluorescent response.

The potassium chloride content used in the dialyzer recipient solution was reduced to 4% because that concentration yielded less "noisy" baselines than the 5% recommended in the Hill procedure.

The combination of filters used here were those available in the Turner filter kit (Turner 110-S39). The secondary filter, color specifi-

*Technicon Instruments Corporation.

cation No. 58, is a narrow-pass filter with a peak at 525 mμ, sufficiently close to the 530-mμ peak determined spectrofluorometrically by Schachter (*2*).

Results obtained by further modification with the use of the aqueous 8-hydroxy-5-quinolinesulfonate reagent, which were reported later by Schachter (*3*), are under investigation; the findings will be reported shortly.

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VOL. 4 (1959) CLINICA CHIMICA ACTA 507-514

SPECTROCHEMICAL DETERMINATION OF MAGNESIUM, CHROMIUM, NICKEL, COPPER AND ZINC IN HUMAN PLASMA AND RED CELLS

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Recently this laboratory[1] reported a spectrochemical procedure for the determination of magnesium, chromium, nickel, copper and zinc in human plasma. It was part of a long-range study of the trace element content of human blood being conducted in co-operation with BYRD, LEAVELL *et. al.* in the Hematology Laboratory of the University of Virginia School of Medicine. The work reported in this communication is a continuation of these studies and includes values for magnesium, chromium, nickel, copper and zinc in human red cells, as well as additional values for these metals in human plasma. All samples of blood were taken from persons believed to be normal in health; a number of the samples were taken from the same individuals at intervals of several weeks to several months in order to determine the variation for the metal concentrations in the sample donors over a period of time. Similar studies are being made on samples taken from diseased persons but these will be reported later in a joint publication with the Hematology Laboratory.

The procedures given in this paper require only about half the overall time required by those previously reported[1] for plasma (35 hours), being 14 h for plasma and 17 h for red cells.

APPARATUS

Spectrograph. Applied Research Laboratories 2-meter grating spectrograph, modified with exterior optics and ignitor[2]. The camera was set so that all five elements could be determined on one film, covering a range from 4200 to 7000 Å. Eighteen spectra were photographed on one film.

Excitation source. Applied Research Laboratories rectifier unit, 0–15 A direct current.

Densitometer. Applied Research Laboratories film projection comparator-densitometer.

Electrodes. National Carbon special spectrographic graphite, 3/16-inch-diameter rod, cut to 1-inch lengths. The sample end of the electrode was drilled to a depth of 1/16 inch with a 3/16-inch drill. The cup thus formed was treated with a drop of paraffin solution in carbon tetrachloride to prevent absorption of the sample by the electrode. The sample electrode is lower and negative. The upper and counter electrode is 1/8-inch in diameter.

Muffle furnaces. Electric furnaces operated by automatic controllers (Wheelco Model 241 P) were used in ashing the samples. The temperature was checked with a thermocouple and was held within 465–475°.

Hypodermic syringes. 50-ml syringes, fitted with special platinum (89%)–ruthenium (11%) alloy needles (J. Bishop and Co., Platinum Works, Malvern, Pa.). The needles are size No. 19, and their collars are heavily gold plated.

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REAGENTS

Ammonium hydroxide. Baker and Adamson, reagent grade. Free of molybdenum and the metals being determined as shown spectrographically.

Hydrochloric and nitric acids. Baker's analysed reagents, distilled in an all-Pyrex distilling apparatus until spectrographic analyses showed the absence of molybdenum and the metals to be determined.

Hydrogen peroxide. Fisher certified reagent, high purity, 30%, with only 0.0001% heavy metals, 0.00002% Fe and free of Mg, Cr, Ni, Cu, Zn and Mo.

Sodium heparin, solid salt. USP, Organon, Inc., Orange, N.J. Qualitative spectrographic analysis of a sample showed it to be free of molybdenum and metals to be determined.

Standard solutions. Solutions of sodium, potassium, calcium, magnesium, chromium, iron, nickel, copper and zinc were made from spectroscopically pure salts or metals (Mathey's "specpure") and were used in the preparation of "synthetic" blood fractions.

Molybdenum trioxide (internal standard). Add 1.500 g of molybdenum trioxide ("specpure") to a 1-l volumetric flask containing 100 ml of concentrated ammonium hydroxide and gently warm on a hot plate till solution is complete. Then add 300 ml triply distilled hydrochloric acid, dilute to the mark with triply distilled water and mix. This solution contains 1 mg Mo per ml.

Triply distilled water. Laboratory distilled water was redistilled twice. Two Pyrex stills in series and automatically controlled were used, the second condenser being fitted with a fused silica tube leading into a large polyethylene bottle protected from dust.

EXPERIMENTAL

A. Cleaning procedure

1. All glassware and polyethylene bottles, etc. used in the analyses should be cleaned in the following manner: (a) Wash thoroughly with detergent and water; (b) Immerse in a 1:1 mixture of concentrated sulfuric and nitric acids for at least one hour; (c) Rinse six times with distilled water, followed by six rinsings with triply distilled water; (d) Dry in an oven at 80°.

2. The needles are cleaned in the same manner as described above except that they are dipped in the acid bath for 10 to 15 sec only. The clean needles and syringes are placed in glass jackets, which have also been cleaned, and are sterilized in an oven at 180° overnight.

B. Synthetic samples and calibration curves

Calibration curves were obtained from synthetic samples whose composition closely approximated that of the blood fraction being analysed (see Table I). These samples were stored in sealed polyethylene bottles and were used periodically for checking and/or recalibration of the standard curves.

C. Preparation of samples

The blood samples must be drawn from the donor in a careful manner in order to avoid contamination. They were drawn as described by THIERS, WILLIAMS AND

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YOE[3] in their work on cobalt, using a 50-ml hypodermic syringe, fitted with a special platinum-ruthenium alloy needle. Sodium heparin (1.6 mg per 50 ml of sample) is placed in the syringe before the sample is drawn so as to prevent coagulation of the blood. Immediately after being drawn, the blood is transferred to a 60-ml polyethylene bottle and the red cells and plasma fractions separated by centrifugation. Samples

TABLE I

DATA ON SYNTHETIC SAMPLES

Element	*Concentrations in p.p.m.* *Cells*	*Plasma*
Sodium	1200	2000
Potassium	400	200
Calcium	20	100
Magnesium	30–96	12–36
Iron	510	1
Chromium	0.024–0.08	0.02–0.08
Nickel	0.020–0.08	0.04–0.125
Copper	0.4–1.4	0.6–2.0
Zinc	4–20	1–7.5

ranging from four to six grams for the cells and from nine to eleven grams for the plasma, are drawn from the bottle with a syringe and are individually transferred to previously weighed 50-ml Erlenmeyer flasks that have been tightly covered with thin polyethylene caps. The flasks are uncapped, reweighed and the sample weights obtained by difference. The samples are dried under a set of 250-W lamps; this requires about 2 h. They are then transferred to a hot plate under another infrared lamp, charred for about one hour and placed in a muffle furnace at 465–475°. The plasma requires about 7 h for ashing; the red cells about 10 h. Only one sample out of sixty required a longer ashing time. "Synthetic" samples containing known amounts of zinc were given the same analysis steps as the plasma and red cells and there was no indication of loss of zinc due to volatilization (see Table III). However, a word of warning is in order because of the danger of local overheating in the case of large samples, due to the heat resulting from oxidation[1, 4, 5].

The samples are removed from the furnace, cooled and to each is added 2 ml of concentrated nitric acid and 0.25 ml of 30% hydrogen peroxide. They are evaporated to dryness under infrared lamps and given successive treatments of 1 ml of nitric acid to insure complete removal of any organic matter left from the ashing. The salts are converted to chlorides by the addition of hydrochloric acid and evaporation to dryness. They are then ready for dilution and spectrographic analysis.

D. Analytical procedure

The chloride salts are dissolved in triply distilled water and made to volume in 5-ml volumetric flasks to which has been added 0.13 ml of the molybdenum trioxide solution as the internal standard. To facilitate dissolving the plasma samples, two drops of hydrogen peroxide were added to the chloride salts. An appropriate volume of sample solution (see Table II) is transferred dropwise with a syringe to a graphite electrode and evaporated to dryness. A soapstone block drilled to hold electrodes serves as a convenient holder; it is placed on a hot plate at 130°.

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The arcing, exposure, and photographic processing conditions are summarized in Table II. After developing the film, the percentage transmission of the analytical lines (all in the second order) of the elements being determined the internal stan-

TABLE II

DATA ON SPECTROGRAPHIC CONDITIONS OF THE METHOD

Spectral region	4200–7000 Å	Emulsion	SA No. 2 film
Slit width	0.04 mm	Development	Eastman Kodak D 19 5 min at 70 °F
Preburn time	none	Fixing	Eastman Kodak, rapid liquid fixer, 5 min duration
Exposure period	35 sec		
Amperage	12 A	Washing	10 min
Electrode gap	6 mm		

	Mg	*Cr*	*Ni*	*Cu*	*Zn*
Analytical lines (2nd order)	2795.53	4254.34	3414.76	3247.54	3345.02
Internal stand lines (2nd order)	2816.15	2816.15	2816.15	3208.83	2816.15
Amount/electrode (cell)	0.22 ml	0.22 ml	0.22 ml	0.05 ml	0.22 ml
Amount/electrode (plasma)	0.35 ml	0.35 ml	0.35 ml	0.03 ml	0.35 ml

dard lines are measured with a densitometer and the concentration of the metals are read off from the calibration curve, prepared by plotting p.p.m. against the ratio of the intensity of the internal standard line to that of the analytical line.

RESULTS

A. Accuracy of the method

A comparison with those reported in the literature indicates that our results are consistent. However, a more rigorous evaluation of the method was desired. This was

TABLE III

DATA ON ACCURACY OF METHOD

Run	Element	*Cells*		*Plasma*	
		Amount added in p.p.m.	*Amount recovered in p.p.m.*	*Amount added in p.p.m.*	*Amount recovered in p.p.m.*
1	Magnesium	40.0	38.7	10.0	9.79
2		70.0	70.6	25.0	26.1
3		100.0	98.8	40.0	40.75
1	Chromium	0.01	0.011	0.01	0.01
2		0.025	0.024	0.025	0.027
3		0.04	0.04	0.04	0.044
1	Nickel	0.05	0.052	0.05	0.054
2		0.10	0.11	0.10	0.10
3		0.20	0.21	0.20	0.19
1	Copper	0.40	0.414	0.50	0.54
2		1.00	1.03	1.00	0.97
3		2.00	1.89	2.00	1.98
1	Zinc	5.00	4.78	1.00	0.98
2		12.00	12.36	3.00	3.06
3		20.00	19.67	5.00	5.13

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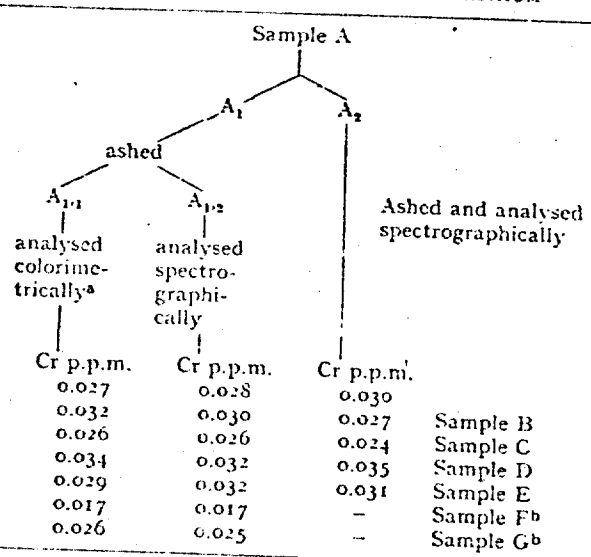
done by preparing "synthetic" samples of the five metals under consideration and analyzing them. The results are shown in Table III. In the case of chromium the method was further checked against a spectrophotometric method recently developed in

TABLE IV

DATA ON ACCURACY OF METHOD FOR CHROMIUM



Cr p.p.m.	Cr p.p.m.	Cr p.p.m.	
0.027	0.028	0.030	
0.032	0.030	0.027	Sample B
0.026	0.026	0.024	Sample C
0.034	0.032	0.035	Sample D
0.029	0.032	0.031	Sample E
0.017	0.017	–	Sample F[b]
0.026	0.025	–	Sample G[b]

[a] Analyses by DWIGHT O. MILLER.
[b] Samples F and G were separated after they had been ashed.

this laboratory[6]. The steps followed in the two methods and a comparison of the results are presented schematically in Table IV. As is noted, the two methods gave satisfactory values for chromium and are in excellent agreement with each other.

B. Precision of the method

A series of samples was analyzed at intervals of three months in order to determine any fluctuation in the standard values obtained from the calibration curves. The results for the blood fractions of one of these samples are shown in Table V. The coefficients of variation are given in Table VI.

TABLE V

DATA ON PRECISION OF THE METHOD

Element	*Blood fraction*	*Run 1 p.p.m.*	*Run 2 p.p.m.*	*Run 3 p.p.m.*	*Average p.p.m.*
Magnesium	Plasma	14.0	16.2	15.0	15.1
	Cells	52.7	58.6	50.2	53.8
Chromium	Plasma	0.024	0.025	0.025	0.025
	Cells	0.017	0.019	0.016	0.017
Nickel	Plasma	0.00	0.00	0.00	0.00
	Cells	0.00	0.00	0.00	0.00
Copper	Plasma	0.58	0.60	0.67	0.62
	Cells	0.59	0.92	1.07	0.99
Zinc	Plasma	2.30	3.00	2.60	2.63
	Cells	8.80	9.20	9.30	9.10

TABLE VI

COEFFICIENT OF VARIATION[a]

Element	Blood fraction	Number of determinations	Average concentration p.p.m.	Coefficient of variation, v
Magnesium	Plasma	39	19.4	0.30
	Red cell	40	64.8	0.37
Chromium	Plasma	39	0.024	0.19
	Red cell	40	0.022	0.31
Nickel	Plasma	39	0.023	1.95
	Red cell	40	0.051	0.72
Copper	Plasma	39	0.99	0.13
	Red cell	40	0.94	0.63
Zinc	Plasma	39	2.70	0.44
	Red cell	40	11.40	0.27

[a] Calculated as follows:

$$v = \frac{1}{C}\sqrt{\frac{\Sigma d^2}{n}}$$

where: d = difference of the determination from the mean.
n = number of determinations.
C = average concentration.

TABLE VII

MAGNESIUM, CHROMIUM, NICKEL, COPPER AND ZINC IN HUMAN PLASMA

Element	Average concentration p.p.m.	Spread	Number of determinations	Method	Investigator
Magnesium	19.4	13.1–37.2	39	Spectrochemical	PAIXAO AND YOE
	20.4	17.0–28.8		Colorimetric	ALBRITTON [7]
	24.0	10.0–42.0	25	Spectrochemical	MONACELLI *et al.* [1]
	22.7			Colorimetric	ORANGE [8]
		17.0–25.0		Spectrochemical	SHIMP *et al.* [9]
Chromium	0.024	0.016– 0.038	39	Spectrochemical	PAIXAO AND YOE
	0.180	0.080– 0.300	25	Spectrochemical	MONACELLI *et al.* [1]
	0.020	0.007– 0.050	17	Colorimetric	KOCH *et al.* [10]
Nickel	0.023	0.00– 0.18	39	Spectrochemical	PAIXAO AND YOE
	0.040	0.01– 0.06	12	Spectrochemical	MONACELLI *et al.* [1]
	0.030	0.01– 0.09		Spectrochemical	KOCH *et al.* [10]
Copper	0.99	0.50– 2.47	39	Spectrochemical	PAIXAO AND YOE
	1.10	0.76– 1.42		Colorimetric	ALBRITTON [7]
	1.20	1.00– 2.10	22	Spectrochemical	MONACELLI *et al.* [1]
	0.92	0.67– 1.30	12	Spectrochemical	KOCH *et al.* [10]
	0.98	0.65– 1.35	58	Colorimetric	KOCH *et al.* [10]
Zinc	2.70	0.48– 4.80	39	Spectrochemical	PAIXAO AND YOE
	3.00	0.00– 6.13		Colorimetric	ALBRITTON [7]
	1.30	0.60– 2.30	14	Spectrochemical	MONACELLI *et al.* [1]
	1.20	0.32– 1.70	58	Colorimetric	KOCH *et al.* [10]
	1.95	1.37– 2.84	100	Colorimetric	WOLFF [11]
	3.50	1.20–11.40	30	Colorimetric	VALLEE [12]
	1.25	0.84– 1.63	62	Colorimetric	VICKBLADH [13]
	1.13	0.72– 1.60	24	Colorimetric	BERFENSTRAM [14]
	0.71	0.35– 0.80		Spectrochemical	SHIMP *et al.* [9]

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C. Discussion

The results obtained in the analyses are grouped separately for plasma and red cells and are listed in Tables VII and VIII. From these results, values for whole blood were calculated based on 55 and 45 volume percents for plasma and cells, respectively.

TABLE VIII

MAGNESIUM, CHROMIUM, NICKEL, COPPER AND ZINC IN HUMAN RED CELLS

Element	*Average concentration p.p.m.*	*Spread*	*Number of determinations*	*Method*	*Investigator*
Magnesium	64.8	26.6 –112.0	40	Spectrochemical	PAIXAO AND YOE
	61.2			Colorimetric	ALBRITTON[7]
Chromium	0.022	0.011–0.041	40	Spectrochemical	PAIXAO AND YOE
Nickel	0.051	0.00 – 0.16	40	Spectrochemical	PAIXAO AND YOE
Copper	0.94	0.28 – 3.5	40	Colorimetric	ALBRITTON[7]
	0.75	0.49 – 1.01	60	Colorimetric	KOCH *et al.*[10]
	0.85	0.21 – 1.97			
Zinc	11.4	3.80 –16.6	40	Spectrochemical	PAIXAO AND YOE
	14.4	9.10 –19.7		Colorimetric	ALBRITTON[7]
	11.8	7.65 –15.1	57	Colorimetric	KOCH *et al.*[10]
	13.0		20	Colorimetric	WOLFF[11]
	14.3		30	Colorimetric	VALLEE[12]

These results are given Table IX. The accuracy and precision of the method are good. The time required to prepare and analyze a sample has been reduced to less than half the time required for the plasma analysis procedure recently reported by this laboratory[1]. About 17 h are required for the red cells.

TABLE IX

MAGNESIUM, CHROMIUM, NICKEL, COPPER AND ZINC IN HUMAN WHOLE BLOOD

Element	*Average concentration p.p.m.*	*Spread*	*Number of determinations*	*Method*	*Investigator*
Magnesium	40.0[a]			Colorimetric	PAIXAO AND YOE
	41.4	36.0–44.4			ALBRITTON[7]
Chromium	0.023[a]				PAIXAO AND YOE
Nickel	0.036[a]				PAIXAO AND YOE
Copper	0.97[a]			Colorimetric	PAIXAO AND YOE
	0.94	0.73– 1.15		Colorimetric	ALBRITTON[7]
	1.06	0.50– 1.88	54		KOCH *et al.*[10]
Zinc	6.64[a]			Colorimetric	PAIXAO AND YOE
	8.80	4.90–12.7		Colorimetric	ALBRITTON[7]
	6.30	4.50– 9.35	54	Colorimetric	KOCH *et al.*[10]
	7.00	5.60– 9.01	30	Colorimetric	WOLFF[11]
	8.80	5.20–14.8	30	Colorimetric	VALLEE[12]
	8.10	6.90– 9.80	18	Colorimetric	VIKBLADH[13]
	6.60	5.60– 7.50	24	Colorimetric	BERFENSTAM[14]

[a] Calculated from the values for plasma and red cells (Tables VII and VIII).

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SUMMARY

Procedures are given for the collection and digestion of human plasma and red cells for the spectrochemical determination of Mg, Cr, Ni, Cu and Zn. Values found for the five metals are compared with those reported in the literature. The accuracy and precision of the method are good. The time required to prepare and analyze a sample has been reduced to less than half (14 h) required for the plasma analysis procedure recently reported by this laboratory. The red cells require about 17 h.

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Hypermagnesemia in Renal Failure

Etiology and Toxic Manifestations

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MAGNESIUM is the fourth most abundant cation in the body. In a 70-kg man there are approximately 1,000 mEq of magnesium in bone, another 1,000 mEq within cells, and 20 mEq in plasma and interstitial fluid. In normal persons all the magnesium absorbed from the intestinal tract is excreted by the kidneys, as is parenterally administered magnesium (1–5). If the dietary intake of magnesium is curtailed, renal conservation of magnesium occurs (3, 6), and if magnesium depletion results from extra-renal losses, magnesium virtually disappears from the urine (3–5, 7).

In renal disease the ability to excrete magnesium may be severely impaired and serum levels may rise (8–20). It has not been certain, however, that these increments are responsible for any of the clinical manifestations of uremia. Observations in several patients with renal failure and hypermagnesemia after the administration of magnesium-containing antacid or laxative medications suggest that such elevations of the serum magnesium may indeed produce severe toxicity. This prompted investigation of the factors involved and an attempted correlation of the clinical manifestations and the serum magnesium level.

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METHODS

CLINICAL OBSERVATIONS

Five patients with renal insufficiency receiving prescribed magnesium medications developed marked hypermagnesemia and severe manifestations of toxicity. Their pertinent clinical and laboratory data are listed in Table 1. Patients 1 and 2 had been given magnesium sulfate parenterally to treat states of presumed magnesium depletion. Patient 3 was given magnesium sulfate orally to induce diarrhea, and Patients 4 and 5 received magnesium hydroxide in antacid preparations. Individual case reports are not included herein but the clinical symptomatology associated with hypermagnesemia in these individuals is listed in Table 1 and summarized in Results. Only the manifestations that clearly have their onset in association with hypermagnesemia are recorded.

Clinical observations were also made in each of 14 patients with chronic renal failure who *ingested* magnesium medications *experimentally* (Table 2) and in eight patients receiving intravenous *infusions* of magnesium (see Methods below). Observations in these subjects are also summarized in Results.

INGESTION OF MAGNESIUM ANTACIDS

Six normal subjects (Table 3) and 14 patients with chronic renal failure (Table 2) ingested 180 ml daily of an antacid containing either magnesium hydroxide or magnesium trisilicate in six equal 30 ml doses at 3-hr intervals on each of 3 consecutive days. The serum was analyzed before and immediately after this period. In several subjects the 24-hr urinary

TABLE 1. Clinical and Laboratory Data in Patients

Patient No.	Clinical Status	Creatinine Clearance (ml/min)	Magnesium Intake (mEq/day)	Magnesium Intake	Serum				
				Parenteral	Mg	Ca	PO_4	Na	K
					mEq/liter	mg/100 ml	mg/100 ml	mEq/liter	mEq/liter
1	[illegible]	12	[illegible]	$MgSO_4$ 10 mEq/g fasts			2.1	145	2.6
				$MgSO_4$ 16 mEq/g IVS	7.5	5.1	2.6	148	3.7
			[illegible]						
				$MgSO_4$ 110 mEq/g IVS for 10.5 days		6.7	5.0	126	3.2
2	[illegible]	50	23 11 26						
				$MgSO_4$ 220 mEq/g ims for 10.5 days	1.1 7.2	7.2	5.0	132 138	5.4 5.0
			21		3.0	7.1		138	5.0
3	[illegible]	30	0	Mg[illegible] Enema			6.8	9.6	122	3.8
			10 17		1.2 5.4	6.6		140	7.3 8.4
4	[illegible]	50	0	$Mg(OH)_2$						
		15	7		9.1 7.6	6.6	4.3 3.4	135 120 121	3.3 5.5 5.7
5	[illegible]	7	0 10	$Mg(OH)_2$	5.0	8.9 8.0	7.1	138	5.3 7.1
			11		5.4	7.6	7.9	130	8.1

excretion of magnesium was determined for 2 or 3 days before the period of administration as well as during and after the period. Only the values for the day immediately preceding the medication and for the third day of ingestion are listed in Tables 2 and 3. The amount and type of food ingested were kept constant during the study. Stools were not analyzed because of the brevity of the periods of study.

PARENTERAL ADMINISTRATION OF MAGNESIUM

Infusions of magnesium sulfate were administered to two normal subjects and to three patients with varying degrees of renal insufficiency. During a flat period 90 mEq of magnesium as the sulfate in 500 ml of isotonic saline were infused (8:30 AM to 12:30 PM) using a constant-infusion pump. In each individual, a control infusion of saline without the magnesium was performed 1 or 2 days prior to the magnesium infusion. On both the control and the infusion days, identical meals were ingested at 8:00 AM, 1:00 PM, and 5:00 PM; hourly urine samples and mid-period sera were obtained from 7:00 AM until 5:00 PM. All specimens were analyzed for magnesium, calcium, sodium, potassium, chloride, phosphate, and creatinine.

The effects of intravenous magnesium sulfate administration were also observed in one patient with idiopathic hypoparathyroidism infused at a similar rate and in two normal subjects infused at a faster rate (167 mEq during 1 hour).

Observations obtained approximately half-hourly during these infusions included: [1] 12-lead electrocardiograms;* [2] measurements of pulse and respiratory rate; [3] blood pressures in the recumbent and upright position (after 5 minutes tilted to 20 degrees from the vertical on a "rocking bed"); [4] testing of patella and biceps deep tendon reflexes; [5] appraisal of the ability to articulate rapidly; and [6] evaluation of the state of consciousness and sub-

* Not performed in the two normal subjects infused with 167 mEq of magnesium.

With Toxic Manifestations of Hypermagnesemia

Serum				Blood		Urine	Electrocardiographic†	Toxicity
Cl	CO_2	Creatinine	pH (art.)‡	Urea N*	Hematocrit Reading			
mEq/liter	*mM/liter*	*mg/100 ml*		*mg/100 ml*	*%*	*c.c./day*		
85	26			62*	43		PR .16, QRS .09, QT_c .40, T waves flat	Following injection of mg., areflexia, apnea, hypotension
80	41	7.0	7.49	100*	48	>1,000		Apnea and death after injection
84	14			60	36		Normal	First course of magnesium caused symptomatology presumably related to magnesium depletion
112	17			36				
109	15	3.2		38		>1,000	Normal rhythm, QRS .16, QT_c .47, T waves inverted	Second course of magnesium followed by arrhythmia, transient asystole, hypotension, coma, decrease in respirations
107	17			74	36		Normal	Improved as serum magnesium level fell
92	17	4.8		90*	27	200	PR .16, QRS .10	
90	8	5.3		225*		0 0	PR .22, P waves flat, QRS .13, T waves tall	P-R prolongation, Coma and death probably related to severe uremia
73	26	4.0		81	30	300		
69	36			130		100		Progressive coma, hypotension, diminished reflexes and respirations, death in cardiac arrest
69	37½	6.6	7.59	135	36	50		
106	15	11.0		90	33		PR .15, QRS .08, QT_c .41	
104	15			110		600	PR .32, QRS .16, QT_c .50, S1 depressed, T waves tall	Progressive drowsiness, confusion, hypotension, dysarthria, difficulty voiding, diminished respirations, P-R prolongation
99	14	12.0		125	33	1,000	Same	Death

* NPN rather than BUN.
† All interval measurements are in seconds.
‡ Given as Maalox ®, 120 to 300 ml daily.
§ Administered as 50% $MgSO_4 \cdot 7H_2O$ as prepared by Lilly & Co.
‖ Arterial pCO_2 30 mm Hg at this time.

jective feelings of nausea, drowsiness and malaise. Gait and coordination were tested at the termination of the infusion. Massage of each carotid sinus for periods of 7 sec during electrocardiographic monitoring was performed before the magnesium infusion and half-hourly during the last 1½ hr of the infusion. This testing was prompted by the experience in Patient 17 (see Results). Only the significant changes occurring during infusion are summarized in Results.

ANALYTICAL METHODS

Serum magnesium was determined by a modified titan yellow method of Garner (22). The normal serum magnesium in this laboratory is 1.7 to 2.1 mEq/liter.* Urines were analyzed after first digesting with nitric and sulfuric acid. Calcium was determined by a method adapted from Elliot (21), using ethylenediaminetetraacetic acid titration, Murexide, and automatic titration on an "EEL" Photoelectric Titrator (manufactured by Evans Electroselenium, Ltd., England). Other determinations were according to methods previously reported from this laboratory (7).

* Certain organic molecules interfere significantly with the determination of magnesium by the titan yellow colorimetric method giving falsely low values unless the samples are first digested to destroy the organic substance (23). To determine whether any factor(s) in uremic sera had a similar effect, recoveries of added magnesium standard were performed using several sera with a urea nitrogen content greater than 200 mg/100 ml. No interference with the accuracy of this method was found.

RESULTS

CLINICAL MANIFESTATIONS ASSOCIATED WITH HYPERMAGNESEMIA

The clinical manifestations of renal failure are complex, and it is difficult to

TABLE 2. Clinical and Laboratory Data in Patients with Renal Disease Ingesting Magnesium-antacids Experimentally

Patient No.	Diagnosis*	Creatinine Clearance	Medication†	Serum Magnesium		Blood Urea N	Serum			Urine Magnesium		Toxicity**	
				Before Antacid	After 3 days		Cr	Ca	PO_4	Before Antacid	Third Day		
		ml/min		*mEq/liter*		*mg/100 ml*				*mg/24 hr*			
6	Chronic glomerulonephritis (b)	30	Maalox	2.0	3.0	36	3.2	8.3	4.6	61	170	Nausea	
7	Nephrosclerosis (a)	9	Maalox	1.8	4.3	116	9.0	5.0	12.0			Sleepy, nausea	M
8	Diabetic glomerulosclerosis (b)	11	Gelusil	2.1	3.4	106	7.0	8.7	6.9	84	136		
9	Chronic glomerulonephritis (b)	29	Maalox	2.8	3.6	75	5.2	9.1	4.8				
10	Diabetic glomerulosclerosis (b)	28	Maalox	2.4	3.0	62	2.4	9.7	5.3				
11	Diabetic glomerulosclerosis (c)	8	Maalox	2.4	2.6	159	11.9	8.0	7.6	65	82		
12	Chronic glomerulonephritis; congestive failure (c)	10	Maalox	2.3	3.9	108	5.6	8.1	5.3			Drowsy	
13	Nephrosclerosis; nephrocalcinosis (a)	8	Maalox	1.7	3.5	100	5.0	7.6	3.3				
14	Amyloidosis (b)		Maalox	2.0	2.7	40		8.6	4.7				
15	Nephrocalcinosis (Milk-alkali) (c)‡	55	Gelusil	2.2	2.5	37	1.5	10.6	3.0	125	300		
16	Nephrocalcinosis (Milk-alkali) (b)	30	Maalox	2.4	3.5	51	3.2	13.0	2.7				
17	Chronic pyelonephritis (a)	8	Maalox§	2.6	4.9	100	7.9	7.3	6.4	50	120	Nausea, drowsiness, malaise; difficult micturition and defecation; muscle twitches; postural hypotension, slight P-R prolongation	
18	Chronic glomerulonephritis (a)	4	Maalox	1.8	3.2	200	16.4	7.9	8.1	32	44	Drowsiness	
19	Adenocarcinoma prostate (c)		Maalox	1.8	3.6	60		8.3	2.7				

* Established by autopsy examination (a), renal biopsy (b) or clinically (c).
† See subscript Table 3.
** Toxic manifestations began during the period of medication and subsided upon stopping the medication as the serum magnesium concentration fell.
‡ Reported in detail elsewhere (21).
§ Similar results were obtained during the ingestion of a comparable amount of magnesium hydrox[illegible]

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TABLE 3. Serum and Urine Magnesium in Normal Subjects Ingesting Antacids

Subject No.	Creatinine Clearance	Antacids*	Serum Magnesium		Urine Magnesium	
			Before Antacid	After 3 days	Before Antacid	Third Day
	ml/min	*150 ml/day*	*mEq/liter*		*mg/24 hr*	
1	110	Maalox	1.9	2.1	122	234
2	120	Maalox	2.1	2.0	87	284
2	120	Gelusil	2.0	2.0	85	400
3	100	Maalox	2.2	2.3		
4	115	Maalox	1.9	2.0		
5	130	Maalox	1.7	1.8	115	270
6	140	Gelusil	2.3	2.5	125	300

* Provides approximately 2.8 g (235 mEq) of magnesium as $Mg(OH)_2$ in Maalox® and 2.9 g (240 mEq) of magnesium as magnesium trisilicate in Gelusil®.

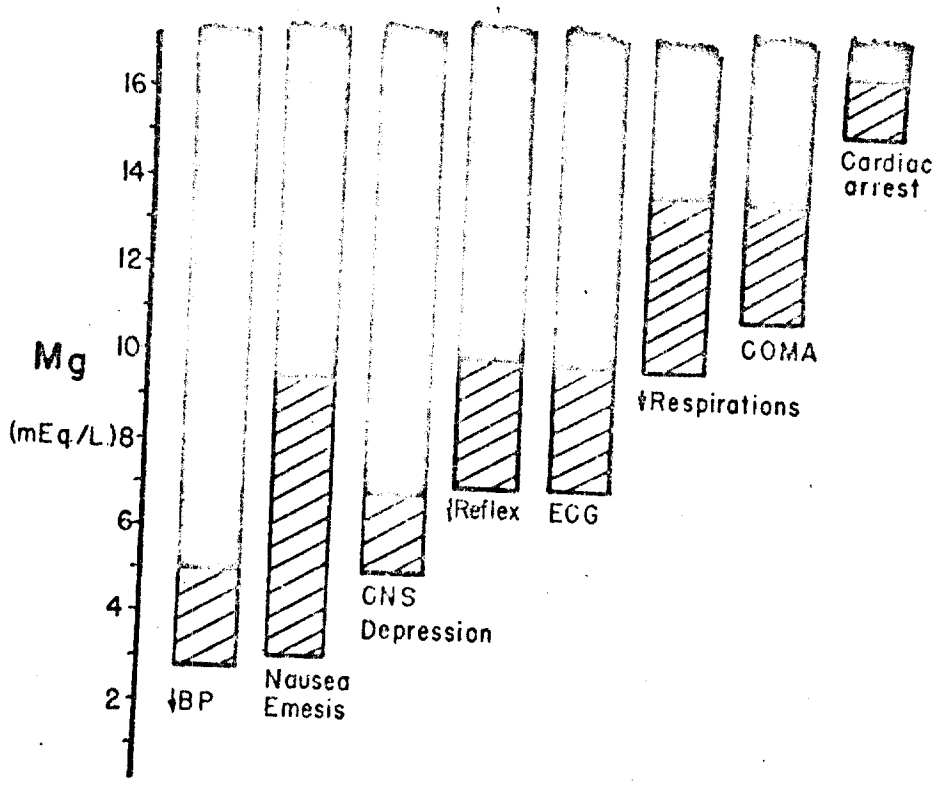

FIGURE 1. Signs and symptoms of hypermagnesemia. The hatched areas represent the variable serum concentrations at which the toxic phenomena may occur during the infusion of magnesium and the solid areas imply uniform occurrence of the phenomena at the concentrations represented. The relationships portrayed represent generalizations from previous studies rather than precise observations. Many of the observations, particularly at the higher serum concentrations, are from experiments in animals (25–35); studies in man have been few (11, 12, 25–28). The importance of other electrolyte concentrations in these relationships has not been evaluated. (See text).

identify precisely those manifestations that are caused solely by an increase in the serum concentration of magnesium. To define such relationships in the patients studied, it is necessary to refer to previous reports making such correlations during experimental hypermagnesemia in man (11, 12, 25–28) and in animals (25–35). Figure 1 portrays these relationships and may serve as a guide in this report. The following manifestations had their onset associated with hypermagnesemia:

Blood Pressure: Hypotension occurred in four of the five patients who developed hypermagnesemia after prescribed doses of magnesium-containing medications (Table 1) at serum concentrations of 5.0 mEq/liter or greater. During the *experimental* ingestion of magnesium hydroxide, one patient (No. 17) had a slight fall in the supine blood pressure but a pronounced lowering in the upright position associated with a serum concentration of 4.9 mEq/liter.

Infusions of magnesium produced hypotension in normal subjects (twice) when serum concentrations of approximately 10 mEq/liter were attained. The patient with hypoparathyroidism (serum calcium 5.7 mg/100 ml) experienced mild hypotension (140/80 to 90/60 mm Hg) when the serum magnesium concentration was 5.9 mEq/liter, but no other patient had any significant change in blood pressure during infusion (serum magnesium up to 6.7 mEq/liter).

Nausea, Vomiting, Malaise: These symptoms occurred in two normal subjects receiving rapid infusions at serum concentrations above 10 mEq/liter but disappeared when the serum concentrations fell below 8 mEq/liter. Only one patient (No. 6) experienced these symptoms at lower concentrations of the serum magnesium; severe nausea and vomiting began during the infusion at serum concentrations of 5 mEq/liter and did not abate until 2 days later when the concentration was 3 mEq/liter. One patient (No. 17) experienced these symptoms during the *ingestion* of magnesium hyroxide at a serum concentration of 4.9 mEq/liter. Other disorders complicate the interpretation of these symptoms in the other patients.

Micturition: Difficulty in initiating urination occurred in several subjects when the serum concentration was 4.5 mEq/liter or greater. A temporary inability to void occurred in the two subjects infused with

TABLE 4. Electrocardiographic Changes During Infusion of $MgSO_4$

Patient	Serum			P-R	Electrocardiogram		
	Mg	Potassium	Ca			Observed Q-T	QT$_c$*
	mEq/liter	*mEq/liter*	*mg/100 ml*	*sec*	*rate/min*	*sec*	*sec*
17†	1.8	4.9	5.7	.16	64	.40	.41
	3.7	4.8	5.3	.21	75	.40	.45
	4.8	4.9	5.6	.26	70	.45	.49
	4.8	5.0	6.3‡	.22		(see text)	
6	2.4	4.5	8.8	.12	80	.37	.43
	6.7	4.5	8.8	.17	80	.37	.43
Hypoparathyroid patient	2.0	3.9	5.9	.13	110	.36	.49
	5.2	3.8	5.5	.17	95	.40	.50
	5.9	3.8	5.7	.17	90	.40	.49

* According to Basset's formula (36).
† Receiving 0.1 g digitalis daily.
‡ Intravenous calcium given after cardiac arrest.

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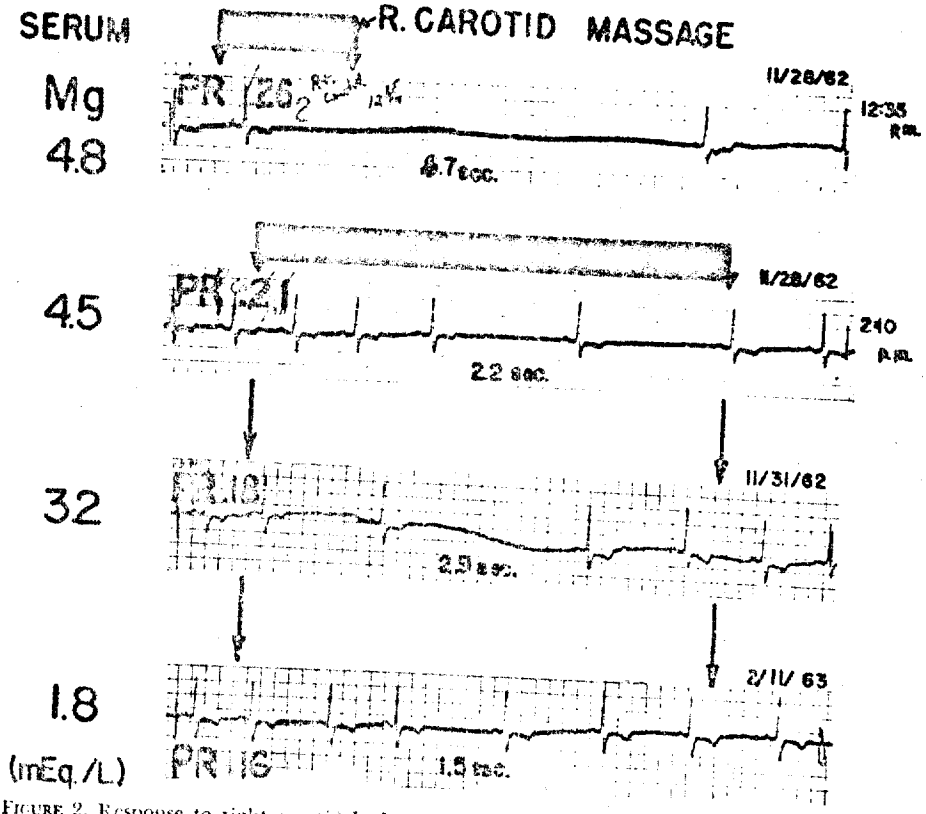

FIGURE 2. Response to right carotid body massage in *Patient 17. Top:* During the hypermagnesemia of magnesium sulfate infusion, 2-sec massage produces cardiac arrest lasting 6.7 sec, followed by pedal escape. The P-R interval had increased from 0.18 sec. to 0.26 sec during the infusion; this E.C.G was taken immediately after an intravenous injection of 7.2 mEq of calcium chloride when the P-R interval measured 0.22 sec. *Second:* Seven second massage later on the same day. *Third:* Three days later. *Bottom:* Same stimulus 2 months later demonstrating less slowing and a shorter P-R interval.

167 mEq; one required catheterization. Patient 17 also experienced difficulty in initiating defecation when the serum magnesium was above 4 mEq/liter.

Central nervous system depression occurred in all individuals with serum magnesium concentrations above approximately 4 mEq/liter. This was characterized by drowsiness, lethargy, slight slurring of speech, an ataxic gait, and a tendency to fall sidewards while standing erect. These manifestations were marked in Patient 6 and in the hypoparathyroid patient.

Coma, associated with diminished or absent deep tendon reflexes and diminished respirations, occurred in one normal subject at serum concentrations above 10 mEq/liter and in Patients 1, 2, and 4, at concentrations greater than 7.5 mEq/liter.

Electrocardiographic Changes and Cardiac Arrest: P-R prolongation occurred in Patients 3, 5, and 17 during hypermagnesemia (*ca.* 5 mEq/liter) resulting from the ingestion of magnesium substances. During infusion, three patients showed changes as recorded in Table 1. Two of these patients were significantly hypocalcemic, and one was receiving digitalis daily.

P Waves: Slight reduction in P wave voltage occurred in Patient 3 and Patient 17 during ingestion of magnesium compounds and in the hypoparathyroid pa-

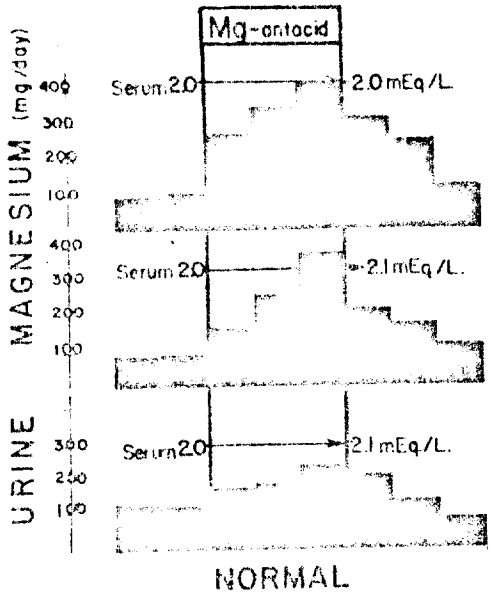

FIGURE 3. Serum and urine magnesium in three *normal* subjects during the ingestion of 180 ml daily of Gelusil® (*top*) or Maalox® (*bottom two*) for 3 days.

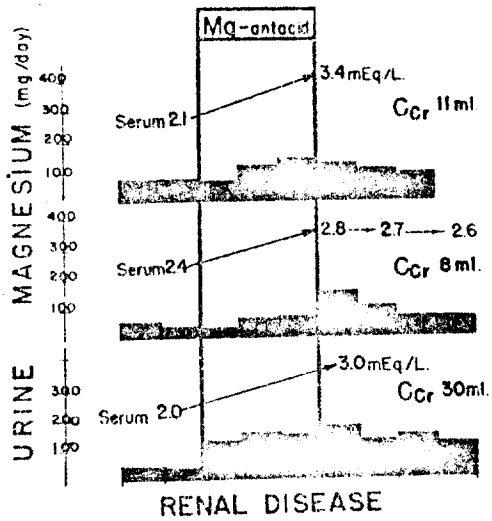

FIGURE 4. Serum and urine magnesium in patient with *renal failure* ingesting 180 ml of Gelusil® (*Patient 8, top*) or Maalox® (*Patient 11, middle, and Patient 6, bottom*) daily for 3 days.

tient during infusion of magnesium sulfate at the height of the hypermagnesemia (serum magnesium, > 5 mEq/liter).

QRS Duration: Nodal rhythm with bundle branch block occurred in Patient 2 when the serum magnesium was 7.2 mEq/liter; normal sinus rhythm and normal conduction returned in association with a decrease in the serum magnesium. QRS prolongation also occurred in Patients 3 and 5 during hypermagnesemia and hyperkalemia. No consistent change occurred in the QRS duration in any subject during the magnesium infusions.

Q-T Interval: The Q-T was increased in Patients 1, 2, and 5. During infusion this increased in Patient 17, remained prolonged in the hypoparathyroid patient and at the upper limits of normal in Patient 6 (Table 4). Hypocalcemia was present in most of these patients.

Cardiac arrest occurred in Patients 1, 2,

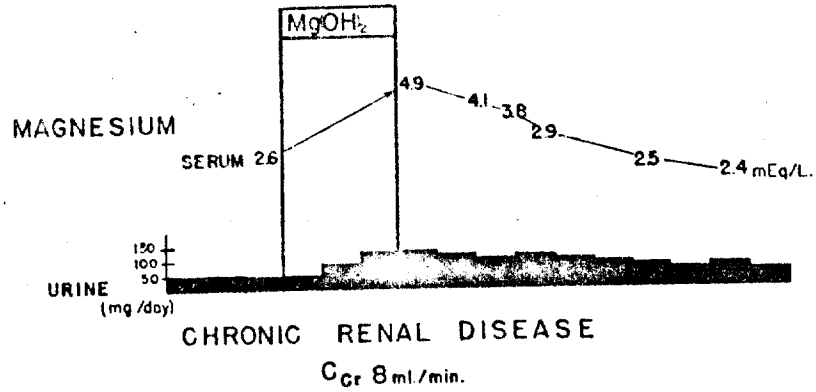

FIGURE 5. *Patient 17* ingesting 180 ml of Maalox® for 3 days.

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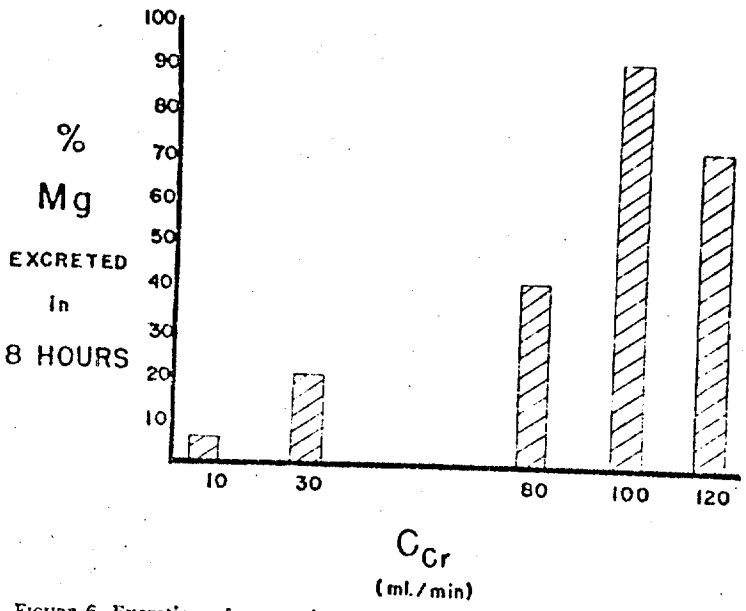

FIGURE 6. Excretion of magnesium after magnesium infusion. The amount appearing in the urine in 8 hr (in excess of the amount during 8 hr on the control day) is expressed as a per cent of the amount infused. The subjects from left to right are *Patient 17*, *Patient 6*, a 64-year-old man without known renal disease or azotemia, and two healthy volunteers.

4, 5, and 17. Hyperkalemia was present in only one of these (Patient 5) at the time of the arrest. In most instances observations were not possible to define the nature of the sudden cessation of heart beat. In Patient 17, however, this occurred during the infusion of magnesium and more detailed observations were possible. The P-R inter-

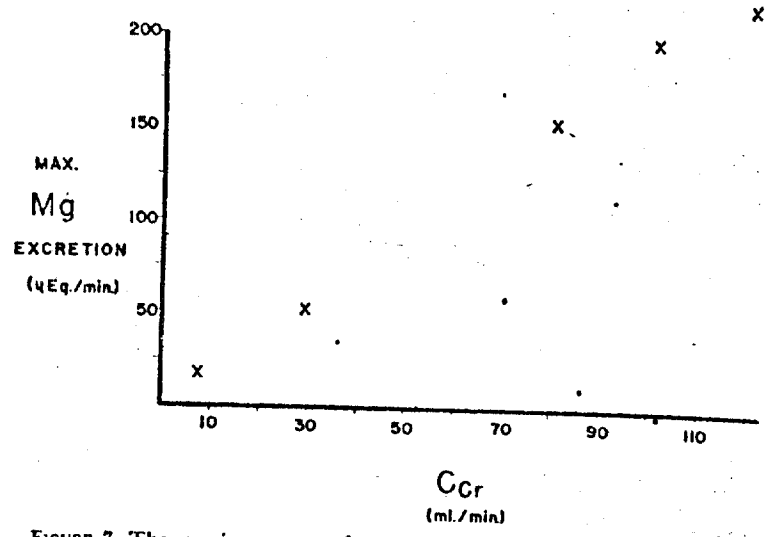

FIGURE 7. The *maximum* rate of renal excretion of magnesium during or immediately after the magnesium infusions for the same subjects portrayed in Figure 6.

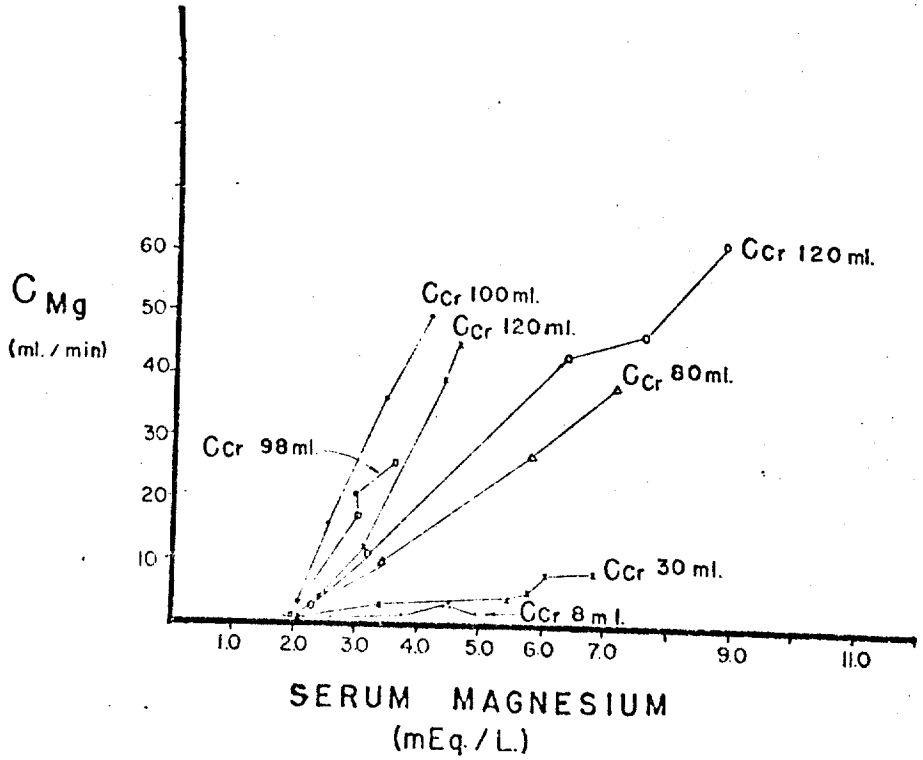

FIGURE 8. The clearance of total serum magnesium during the infusion of magnesium sulfate in subjects with varying filtration rates. In addition to the five subjects portrayed in Figures 6 and 7, the data in one subject with hyperparathyroidism (C_{cr} 98 ml) receiving 54 mEq of magnesium in 4 hr (pre-operation) is shown and the data in one normal subject receiving 167 mEq during 1 hr is shown (C_{cr} 120 ml to the right). The clearance of magnesium is reduced at all serum magnesium levels according to the severity of impairment in C_{cr}. Ultrafiltration of sera was not performed on these samples; it is improbable that increased binding or complexing (19) during infusion could account for the impaired clearances observed.

val had lengthened from 0.16 sec to 0.26 sec toward the end of the infusion period when the patient suddenly turned his head to the left, announced he was going to faint, became unconscious, and had a tonic-clonic seizure during an 8-sec period of cardiac arrest. The infusion was stopped and calcium chloride (7.2 mEq) was injected intravenously. Several minutes later, very light and brief (2-sec) massage of the right carotid sinus reproduced the asystole (Figure 2) and syncope. Further testing was withheld until later in the afternoon of the same day whereupon longer (7-sec) massage produced less slowing and no syncope. Testing at later dates showed that massage would produce only slight slowing of cardiac activity. These data suggest that the induced hypermagnesemia, albeit moderate, had produced a decreased cardiac rhythmicity and conductivity severe enough to result in transient asystole.

GASTROINTESTINAL ABSORPTION OF MAGNESIUM

The effects of ingesting magnesium antacids on the serum concentrations and urinary excretion of magnesium are listed in Table 2 (patients with renal disease) and Table 3 (normal subjects). Figures 3 to 5 portray these results in several subjects. The urine magnesium excretion increased in all subjects. This was significantly greater in the normal subjects than

in the patients with renal disease. Each of the latter subjects had a significant increase in the serum magnesium concentration during this period. These data are taken to represent increased gastrointestinal absorption of magnesium during the ingestion of these antacids.

RENAL EXCRETION OF MAGNESIUM

The lesser daily urinary excretion of magnesium in patients with renal disease (Table 2 and Figures 4 and 5) as compared with normal subjects (Table 3 and Figure 3) despite greater serum concentrations suggests an impairment in the capacity to excrete magnesium. This was confirmed during the magnesium infusion studies (Figures 6 to 8); patients with renal disease have an impairment in the capacity to excrete magnesium that is roughly proportional to the impairment in creatinine clearance.

Discussion

Previous reports (8, 11–13, 15) have not proved conclusively that hypermagnesemia in renal failure produces toxic manifestations. The data just reviewed provide the strongest evidence thus far in support of this possibility. The manifestations observed correspond to those portrayed in Figure 1 and showed a close temporal relationship to the administration of magnesium and to the elevations of the serum magnesium. In several instances (Patients 2, 6, and 17), the manifestations disappeared in association with decreasing concentrations of the serum magnesium. In Patient 17, experimental production of hypermagnesemia produced similar manifestations on three occasions, twice following the ingestion of magnesium hydroxide and once during the infusion of magesium sulfate. It is believed that these observations are sufficient for the present to make further testing unnecessary; moreover, the severity of some of the manifestations makes it unwise to pursue experimentation at higher serum concentrations in man.

Certain facts regarding the observations in these patients deserve emphasis. The serum concentrations at which toxic manifestations appeared were considerably lower than the concentrations associated with these manifestations in the normal subjects of this study and as reported in other studies during experimental hypermagnesemia (25–35, 37, 38). This is particularly true of the electrocardiographic abnormalities (P-R, QRS, and Q-T prolongation, diminished P wave amplitude, ST depressions and tall T waves) reported to occur only at serum levels greater than 8 mEq/liter, and of cardiac arrest in animals occurring at much higher concentrations (17, 20, 25, 26, 28, 30–33, 35, 37, 38). Most previous testing was carried out in normal subjects or animals; patients with advanced renal failure differ in many respects, frequently manifesting hypocalcemia, hyperphosphatemia, acidosis, hyperkalemia, and hypertension and commonly are receiving medications such as digitalis. It is possible that any of these factors might influence the nature or severity of the manifestations produced by the hypermagnesemia; in particular, factors such as hyperkalemia (17, 20), hypocalcemia (39, 40), and digitalis (41, 42) *might* augment the cardiac manifestations of hypermagnesemia. For the present, it should be recognized that significant toxicity *may* occur at relatively low serum concentrations.

The precise manner in which magnesium excess produces the manifestations of toxicity is not well-understood. It is known that magnesium functions as an essential cofactor in many enzyme reactions and that these reactions may function poorly under experimental conditions of severe excess or deficit (43, 44). Whether deficits or excesses affect these enzyme reactions in *clinical* disorders, however, is not completely clear. It has been demonstrated experimentally *in vitro* that increased magnesium concentrations can reduce neuromuscular irritability and that calcium may

antagonize this inhibition (15–19). It is possible that some of the manifestations of hypermagnesemia (drowsiness, ataxia, dysarthria, hyporeflexia, decreased respirations, and coma) are caused by depressed neuromuscular irritability. This might be due to a direct effect on cellular or cell-membrane function or be mediated by enzyme reactions which regulate the concentration of acetycholine (17–19). Considerable investigation is required before the clinical manifestations of magnesium excess or deficit can be explained.

The present report confirms previous studies demonstrating a limitation in the capacity to excrete magnesium in patients with renal failure (50–52). While it has been recognized that magnesium may be absorbed from ingested laxatives (8–10, 53) and antacids (54), the potential danger of these medications in patients with severely restricted renal function has not been fully appreciated. It is of clinical importance to know at what degree of renal impairment and under what conditions of magnesium intake one should exercise caution in prescribing these medications. While not all of the answers are available, Figure 9 portrays some relationships upon which to guide therapy. Under conditions of ordinary dietary intake of magnesium, increased concentrations of the serum magnesium do not occur until the filtration rate is less than approximately 30 ml/min. At lower rates of filtration, increased concentrations occur but generally do not exceed 4 mEq/liter; serious toxicity probably does not result from these concentrations. Greater concentrations have been reported, particularly during acute renal failure, and while it is possible that increased tissue catabolism and sudden acidosis (55–57) may shift tissue magnesium into the intravascular compartment, most studies have not specifically excluded excess exogenous intake of magnesium in the etiology. Such loads may indeed augment the hypermagnesemia as portrayed in Figure 9. In this study the periods of antacid ingestion were brief and the results of greater amounts or longer periods of ingestion are not known. It is significant, however, that Patient 1 became symptomatic after only 3 days (180 ml of Maalox® daily) and Patient 5 after approximately 10 days (120 ml of Maalox daily).

From the present studies some crude lines can be drawn for the use of antacids in patients with renal failure. In the presence of severely reduced renal function (GFR, < 20 ml/min, BUN, > 70 mg/100 ml, serum creatinine, > 4 mg/100 ml, anuria or marked oliguria), the laxative or antacid preparation selected should probably be free of magnesium. Calcium compounds share the same limitation in renal excretion (21), and hypercalcemia should be watched for if calcium compounds are used. There are as yet no known dangers in using the antacid compounds which contain only aluminum compounds.

When extra-renal *magnesium depletion* (4, 5, 7, 44, 58, 59) occurs in the presence of severe renal insufficiency, replacement magnesium therapy should be cautious. Recommended doses for initial therapy (44, 58) probably should be reduced to approximately 0.2 mEq/kg/hr for the initial 4- to 6-hr period of infusion and 1 mEq/kg for the first day. Therapy should be guided by serum determinations of magnesium and by careful observation for signs of toxicity, as outlined above. Once restoration of the body magnesium is attained, as estimated by the serum concentration, then balance may be maintained by providing as little as 2 to 5 mEq daily for such patients unless sources of loss other than renal (vomiting, diarrhea, draining wounds, or fistulas) are present.

Treatment of magnesium excess will depend upon the severity of the manifestations. Calcium should be given whenever serious toxicity is present (absent deep tendon reflexes, decreased respirations, coma or cardiac arrest); 5 to 10 mEq as calcium

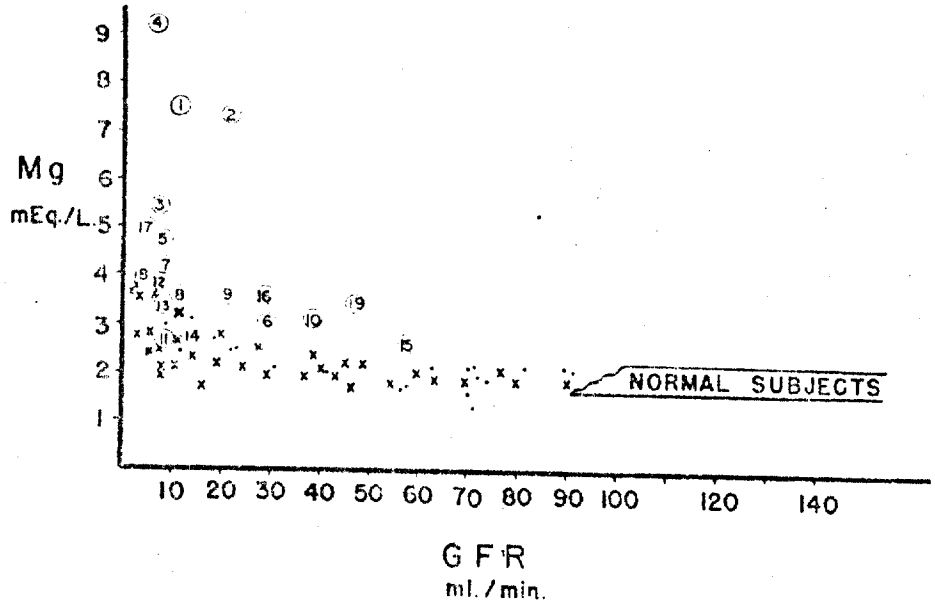

FIGURE 9 Concentrations of the serum magnesium in patients with varying glomerular filtration rates (GFR). The dots represent patients reported by Robinson et al. (14). The crosses represent patients studied by the author who were *not* receiving magnesium other than that contained in the diet, while the circles represent the patients of Tables 1 and 2 who *were receiving excess magnesium* orally or parenterally. The range for normal subjects is shown at the right. The GFR was estimated by C_{In} in the report by Robinson et al. and by C_{Cr} in this report. See text for discussion.

chloride or calcium gluconate should be injected intravenously at a slow rate. The intake of magnesium should be stopped. Magnesium may be removed by hemodialysis (16, 55, 60) or peritoneal dialysis (60); the present data are insufficient to evaluate the requirement for these measures in addition to those mentioned above. If the intake is stopped, renal excretion (and perhaps redistribution) of magnesium will ordinarily allow reduction of the serum concentration; this may, however, require a week or two if the excess is marked.

Summary and Conclusions

1. Data are presented from studies in five patients with severe renal failure who developed marked hypermagnesemia after the administration of magnesium. In two patients, magnesium was given parenterally to treat a presumed state of magnesium depletion, and in the others antacid or laxative medications represented the source of excess magnesium intake.

2. Other patients with renal insufficiency were made hypermagnesemic experimentally by the ingestion or infusion of magnesium compounds. The manifestations in all of these patients were quite similar; they included nausea, vomiting, malaise, hypotension, drowsiness, difficulty in voiding and defecating, dysarthria, ataxia of gait, decreased reflexes and respirations, coma, electrocardiographic changes, carotid sinus sensitivity, and cardiac arrest.

3. Various aspects of the clinical syndrome are discussed and recommendations have been proposed for treating conditions of magnesium depletion and magnesium excess in patients with renal failure.

4. The following conclusions can be drawn regarding etiology: Severe renal disease limits the capacity to excrete magnesium. This limitation is roughly propor-

tional to the impairment in glomerular filtration rate.

Magnesium contained in laxative and antacid medications, previously considered "nonreactive" or "nonabsorbable," may be absorbed to a significant degree. Patients with severe renal failure, ingesting customary doses of standard preparations, may sustain significant elevations of the serum magnesium with manifestations of toxicity in as little as 3 days.

Acknowledgment

Appreciation is expressed to the Misses Miriam Halpin, Regina McLean, and Rosalie Gardner for their technical assistance in this study and to Dr. Maurice B. Strauss for his helpful suggestions in the preparation of the manuscript.

Sumario in Interlingua

Esseva studiate cinque patientes con sever dysfunction renal qui disveloppava marcate grados de hypermagnesiemia post le administration de magnesium. In duo, magnesium esseva administrate per via parenteral como tractamento de un assumite stato de depletion de magnesium. In le altere tres, medicationes antacide o laxative representava le fonte del excessive ingestion de magnesium.

Altere patientes con dysfunction renal esseva rendite hypermagnesiemic a base experimental per le ingestion o infusion de compositos de magnesium. Le manifestationes in omne iste patientes esseva multo simile. Illos includeva nausea, vomito, malaise, hypotension, somnolentia, difficultates de vacuation e de defecation, dysarthria, ataxia del ambulation, declino del reflexos e del respiration, coma, alterationes electrocardiographic, sensibilitate de sinus carotidic, e arresto cardiac. Esseva interprendite le essayo de correlationar iste manifestationes con le nivello de magnesium in le sero. Es facite recommendationes pro le tractamento de conditiones de depletion de magnesium e de excesso de magnesium in patientes con dysfunction renal.

Le sequente conclusiones es formulate con respecto al etiologia. Un sever morbo renal restringe le capacitate de excerner magnesium. Iste restriction es plus o minus proportional al defectivitate del proratas de filtration glomerular.

Magnesium in medicationes antacide e laxative, previemente reguardate comó "non-reactive" o "non-absorbibile", pote esser absorbite a grados significative. Patientes con sever dysfunction renal, quando illes ingere doses costumari de preparatos standard, pote experientiar elevationes significative del magnesium seral con manifestationes de toxicitate in non plus que tres dies.

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French Translation

The influence of magnesium on experimental cancer

by M.P. Serbescu (from Bucharest)

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In many communications presented to The Academy of Medicine and in The Cancer Bulletin Prof. P. Delbet and his co-workers have endeavored to demonstrate that the halogen salts of magnesium have both a preventive and curative effect on cancer. The experiments and statistics which have been employed in support of this thesis do not show the efficacy of the proposed treatment in a definitive manner. It does not seem accurate to generalise results deduced from a very small number of studies on experimental animals and from these to infer positive conclusions on the subject of cancer therapy.

The animals with which one induces experimental tar cancer have a natural tendency for spontaneous recovery as soon as the cause of the lesion disappears. From the anatamo-physiological and histological point of view experimental cancer and natural cancer are identical; it differs in its evolution which is very much slower and less malignant; furthermore except with rare exceptions, unlike natural cancer, it does not show a tendency for generalisation. Almost all the animals with which tar cancer is induced die of poisoning from the tar and rarely owing to the generalisation of the cancerous lesion.

We propose to determine the influence of magnesium on animals with whom we have induced experimental cancer by means of tar. We took two groups of rabbits, 30 animals in each; one group was treated with magnesium

the other served as controls. The animals were put in metallic cages full of hay, in pairs. The diet given daily was made up of bread and alfalfa, in the fall we replaced the alfalfa with beetroot.

We immediately autopsied the dead animals to account for the cause of death; after each decease the cages were sterilised by means of a Bunsen burner flame. Every dead rabbit was then replaced in the cage by a living rabbit. In this way, we experimented on a total of 123 rabbits; 75 were treated with magnesium, the other 48 served as controls. All the animals were painted three times a week in the inside of both ears with tar oil. We gave the first group of rabbits every day except Sunday, 20 mg/kg BW of magnesium. The magnesium was weighed as the crystalline sulfate and dissolved at a concentration of 10 g of metal per liter of redistilled water. The solution was introduced directly into the animals' stomach by means of a rubber probe (Nelaton). To wash down the traces of solution which adhered to the walls of the tube of the probe, we washed the tube with a double amount of distilled water which lowered the magnesium in the animals' stomach. The second group of rabbits were given only the usual food. The animals were very carefully examined immediately after their death; in particular the digestive tract and the ganglionic system were very thoroughly examined.

For the histological examination of the tumors we followed Prof. P. Massons' technique. Immediately after death the skin as well as the induced lesion in the animal's ear, were sectioned with a razor blade and fixed. For fixative we used piroformol. The imbedding and fixing were done respectively in paraffin and in gelatin; on the other hand the staining was carried out by Masson's three color process.

The results of these experiments are summarized as follows:

(1) It was noted the rabbits with the highest mortality were those treated with magnesium: in the first group only one rabbit lived 157 days; at that time it was killed for other research. In the second group, we killed for the same reason 2 rabbits after 197 days, three rabbits after 138 days, finally three rabbits after 105 days of the experiment.

(2) The majority of the rabbits which received magnesium were dead before 40 days without our having observed any cancerous lesions, on the other hand, all those who survived after this period of time developed cancer in a more or less evolved form. On the contrary, among the animals which did not receive magnesium in their food, some lived 73 days without having a cancerous lesion, those which lived a much longer time developed cancer.

So, the animals treated with magnesium developed cancer more easily, likewise they had a higher mortality than that of the animals which did not absorb this element.

M. Pierre Delbet:

If I have correctly understood the report is entitled "Activity or role of magnesium" I protest this title. The author performed these experiments with magnesium sulfate. Whether these experiments warrant any conclusions, they would apply solely to magnesium sulfate.

For myself, I use the word magnesium in short when it concerns nutrition because I do not know in what form this metal is found in food. But there is every reason to believe it is in an assimilable form. In my experiments I use the halogen salts.

I have maintained and I continue maintaining that the halogen salts of magnesium exert a restraining action on canceration. As for the sulfate, I am certain that it cannot have the same effect for two reasons.

First I have explained in this very place that magnesium sulfate does not acidify urine, rather it has a tendency to make it alkaline. Magnesium chloride and in particular the mixture of halogen salts I use, on the contrary, acidify the urine and reduce the pH and this is a fact to which I attach a great deal of importance.

Furthermore, and this is the second reason the sulfate and oxide of magnesium are less well absorbed than the chloride. Taylor and Winter have shown this with very accurate experiments. Lieben has shown that in order to obtain basal anesthesia by the enteric route, it is necessary to use the chloride and not the sulfate, which produces good results by the intravenous route. According to Neuwirth and Wallace when magnesium sulfate or lactate are administered by the gastric route, the level of magnesium in the serum never reaches the concentration of 5 mg/100 needed to obtain reduced pressure. So the sulfate is poorly absorbed. The author of the experiments which have just been reported possibly only succeeded in purging his animals. If that was so, one understands why a daily purge, except Sundays, for some months, would not have increased their resistance. I tried to give magnesium to animals by the gastric route using a probe to inject them with a solution of halogen salts of magnesium.

I quickly abandoned it. It was a question of the guinea pigs. Many regurgitated the solution, possibly because the probe did not penetrate the stomach. On the other hand, catheterisation is not easy with animals which defend themselves. Although I do not use soft probes, it seems to me that catheterised animals have some difficulty in swallowing which disturbs their feeding. Possibly rabbits catheterised for several months had some difficulty feeding themselves.

There are only two ways to give magnesium to laboratory animals under favorable conditions: that is, either to give them food specially enriched with magnesium or to inject them with an isotonic solution of halogen salts of magnesium.

M. Braier (of Buenos Aires) proceeded with injections: He repeated my experiments and he obtained exactly the same results as I did. Moreover, he added a new idea. He was concerned with the metabolism of fats and carbohydrates. For that he determined the ratio $\frac{C}{N}$ in the animals' urine. He found that the ratio becomes lowered in the control cancerous rats, while it remains normal in the magnesium treated cancerous rats. This finding seems to me of great interest. The poor utilisation of fats and carbohydrates is possibly a factor in cancerous cachexia, and in this way the real revival observed in some very cachexic cancerous animals when they are given a large dose of halogen salts of magnesium is explained.

suspects à indice Vernes normal et anormal est très variable; cependant, chez les malades libres, les indices Vernes normaux sont très fréquents, et cela, d'autant plus qu'ils sont plus jeunes.

Les lésions cliniques présentées par ces malades au stade de début de l'infection sont le plus souvent constituées par de simples macules anesthésiques et achromiques chez les gens de couleur. Toutes les recherches bactériologiques sont généralement négatives; et l'imprégnation lépreuse se traduit seulement par des modifications caractéristiques de l'équilibre protéique qui suffisent à authentiquer le diagnostic de lèpre chez les malades qui en présentent déjà les stigmates.

Conclusions. — Certes, la réaction proposée comme élément de diagnostic n'est pas spécifique, elle n'est pas non plus empirique, c'est une réaction pathogénique dont l'interprétation ne saurait se passer d'un contrôle clinique rigoureux. Cette réserve faite, nous pensons qu'elle est appelée à rendre les plus grands services, dans un pays d'endémicité, pour dépister les lépreux. Pour notre part, sur 90 lépreux reconnus actuellement dans notre service, 39 seulement ont montré des B. Hansen dans le mucus nasal ou dans une biopsie; chez tous les autres, le diagnostic de lèpre a été affirmé par l'existence des modifications de l'équilibre protéique associées aux particularités des lésions cliniques.

L'influence du magnésium sur le cancer expérimental,

par M. **P. Serbescu** (de Bucarest).

(Présentation faite par M. **Gabriel Bertrand.**)

Dans plusieurs communications présentées à l'Académie de Médecine et dans le *Bulletin du Cancer*, le professeur P. Delbet et ses collaborateurs ont cherché à démontrer que les sels halogénés du magnésium ont une action préventive et même curative sur le cancer. Les expériences et les statistiques qui ont été apportées à l'appui de cette thèse ne montrent pas d'une manière absolue l'efficacité de la cure proposée. Il ne nous paraît pas rigoureux de généraliser les résultats déduits d'un très petit nombre d'essais sur les animaux mis en expérience et d'en déduire des conclusions définitives au sujet de la thérapie du cancer.

Les animaux chez lesquels on provoque expérimentalement le cancer du goudron ont une tendance naturelle à la guérison spontanée aussitôt que disparaît la cause qui a déterminé la lésion. Le cancer expérimental est au point de vue anatomo-pathologique et histologique identique au cancer naturel; il en diffère par son évolution qui est beaucoup plus lente et moins maligne; d'autre part, sauf quelques rares exceptions, il ne manifeste pas, comme le cancer naturel, de tendance à la généralisation. Les animaux chez lesquels on provoque le cancer du goudron

meurent presque tous de l'intoxication par le goudron et rarement à cause de la généralisation de la lésion cancéreuse.

Nous nous sommes proposé de déterminer l'influence du magnésium sur des animaux chez lesquels nous provoquions le cancer expérimental à l'aide du goudron. Nous avons pris deux lots de lapins, de 30 animaux chacun; un des lots a été traité par le magnésium, l'autre servant de témoin. Les animaux ont été placés deux par deux dans des cages métalliques remplies de foin. La nourriture donnée chaque jour était composée de pain et de luzerne; en automne, nous avons remplacé la luzerne par la betterave.

Sur les animaux morts, nous avons immédiatement pratiqué l'autopsie pour définir la cause de la mort; après chaque décès les cages étaient stérilisées au moyen de la flamme du bec Bunsen. Chaque lapin mort était alors remplacé dans la cage par un lapin vivant. De la sorte, nous avons expérimenté sur un total de 123 lapins : 75 ont été traités avec le magnésium, les 48 autres ont servi de témoin. Chez tous les animaux on a badigeonné intérieurement les deux oreilles, trois fois par semaine, avec du goudron de houille. Au premier lot de lapins nous avons donné chaque jour, sauf le dimanche, 20 milligrammes de magnésium par kilogramme d'animal. Le magnésium était pesé à l'état de sulfate cristallisé et dissout à la concentration de 10 grammes de metal par litre d'eau redistillée. La solution était introduite directement dans l'estomac de l'animal à l'aide d'une sonde de caoutchouc (Nélaton). Pour entraîner les traces de solution qui adhéraient aux parois du tube de la sonde, nous avons lavé ce tube avec une quantité double d'eau distillée, ce qui diminuait la concentration en magnésium dans l'estomac de l'animal. Le deuxième lot de lapins n'a reçu que la nourriture habituelle. Les animaux étaient examinés très attentivement aussitôt après leur mort : en particulier, le tube digestif et le système ganglionnaire ont fait l'objet d'un examen approfondi.

Pour l'examen histologique des tumeurs nous avons suivi la technique du professeur P. Masson. La peau ainsi que la lésion provoquée à l'oreille de l'animal étaient, immédiatement après la mort, sectionnées avec une lame de rasoir et soumises à la fixation. Comme fixateur nous avons utilisé le picroformol. L'inclusion et le collage ont été faits respectivement à la paraffine et à la gélatine; d'autre part, les colorations ont été effectuées par le procédé trichrome de Masson.

Les résultats de ces expériences se résument de la manière suivante :

1° On observe que la mortalité la plus forte se produit chez les lapins traités avec du magnésium : dans le premier lot, 1 lapin seulement a vécu cent cinquante-sept jours; à cette date nous l'avons tué pour d'autres recherches. Dans le deuxième lot, nous avons tué, pour le même but, 2 lapins après cent quatre-vingt-dix-sept jours, 3 lapins après cent trente-huit jours, enfin, 3 lapins après cent cinq jours d'expérience.

2° La plupart des lapins qui ont reçu du magnésium sont morts avant quarante jours sans que nous ayons pu observer de lésions cancéreuses; par contre, tous ceux qui ont survécu après ce délai ont été atteints du cancer sous une forme plus ou moins développée. Au contraire, parmi les animaux qui n'ont pas reçu de magnésium dans leur nourriture, certains ont vécu soixante-treize jours sans avoir de lésion cancéreuse, ceux qui ont vécu plus longtemps ayant été atteints du cancer.

Donc, les animaux traités par le magnésium ont été plus facilement atteints par le cancer; également leur mortalité a été plus grande que celle des animaux qui n'ont pas absorbé cet élément.

M. Pierre Delbet : Si j'ai bien entendu, la communication est intitulée « Action ou rôle du magnésium ». Je proteste contre ce titre. L'auteur a fait des expériences avec le sulfate de magnésium. Si ces expériences légitiment des conclusions, ces dernières s'appliquent uniquement au sulfate de magnésium.

Pour ma part, j'emploie le mot magnésium tout court quand il s'agit d'alimentation, parce que je ne sais pas sous quelle forme se trouve ce métal dans les aliments. Mais il y a tout lieu de penser qu'il est sous une forme assimilable. Dans mes expériences, j'emploie les sels halogénés.

J'ai soutenu et je soutiens toujours que les sels halogénés de magnésium exercent une action frénatrice sur la cancérisation. Quant au sulfate, je suis bien sûr qu'il ne peut pas avoir la même action, et cela pour deux raisons.

D'abord j'ai exposé ici même que le sulfate de magnésium n'acidifie pas l'urine, il a plutôt tendance à l'alcaliniser. Au contraire, le chlorure de magnésium, et particulièrement le mélange de sels halogénés que j'utilise, acidifie l'urine, abaisse le *p*H et c'est un fait auquel j'attache une très grande importance.

En outre, et c'est la seconde raison, le sulfate de magnésium et l'oxyde sont moins bien absorbés que le chlorure. Taylor et Winter l'ont montré par des expériences très précises. Lieben a montré que pour obtenir par voie entérale ce qu'on appelle l'anesthésie de base, il faut employer le chlorure et non le sulfate, qui donne de bons résultats par voie intraveineuse. D'après Neuwirth et Wallace, quand le sulfate ou le lactate de magnésium sont administrés par voie stomacale, le taux du magnésium n'atteint jamais dans le sérum la concentration de 5 milligrammes p. 100 nécessaire pour obtenir la dépression. Le sulfate est donc mal absorbé. L'auteur des expériences qui viennent d'être rapportées n'a peut-être réussi qu'à purger ses animaux. S'il en était ainsi, on comprendrait qu'une purgation tous les jours, sauf le dimanche, pendant des mois, n'ait pas augmenté leur résistance.

J'ai essayé de magnésier des animaux par voie gastrique en leur

injectant une solution de sels halogénés de magnésium au moyen d'une sonde. J'y ai rapidement renoncé. Il s'agissait de cobayes. Beaucoup régurgitaient la solution, peut-être parce que la sonde ne pénétrait pas dans l'estomac. D'autre part, le cathétérisme n'est pas facile sur des animaux qui se défendent. Bien que je ne me sois servi que de sondes molles, il m'a semblé que les animaux cathétérisés avaient quelque peine à déglutir, ce qui troublait leur alimentation. Peut-être les lapins cathétérisés pendant des mois avaient-ils quelque peine à s'alimenter.

Il n'y a que deux moyens de magnésier des animaux de laboratoires dans de bonnes conditions : c'est, ou bien de leur donner des aliments particulièrement riches en magnésium, ou bien de leur injecter une solution isotonique de sels halogénés de magnésium.

M. Braier (de Buenos Aires) a procédé par injections : il a répété mes expériences et il a obtenu exactement les mêmes résultats que moi. De plus, il a ajouté une notion nouvelle. Il s'est préoccupé du métabolisme des graisses et des hydrates de carbone. Pour cela il a déterminé le rapport $\frac{C}{N}$ dans l'urine des animaux. Il a constaté que ce rapport s'abaisse chez les rats cancérisés témoins, tandis qu'il reste normal chez les rats cancérisés magnésiés. Cette constatation me paraît d'un haut intérêt. La mauvaise utilisation des graisses et des hydrates de carbone est peut-être un facteur de la cachexie cancéreuse et ainsi s'expliqueraient les véritables résurrections que l'on observe chez certains cancéreux très cachectiques, quand on leur donne des sels halogénés de magnésium à haute dose.

Sur onze cas de cirrhose traités par la diathermie,

par MM. **Carrière** et **Martin** (de Lille).

Nous avons traité par la diathermie transhépatique, méthode préconisée par Ph. Pagniez, 10 cas de cirrhose avec ascite et 1 cas de cirrhose hypertrophique sans ascite. Les malades étaient soumis, suivant la technique habituelle, au passage d'un courant transabdominal de 1.500 à 2.500 milliampères pendant quinze à vingt minutes.

Dans 6 cas le traitement se montra dépourvu d'efficacité ou n'eut qu'une action très passagère sur la diurèse. Il s'agissait de cirrhoses atrophiques d'origine éthylique avec volumineuses ascites, cirrhoses arrivées à une période déjà avancée de leur évolution. Ces malades n'avaient d'ailleurs nullement réagi aux autres traitements auxquels ils avaient été antérieurement soumis.

Dans 2 cas, nous avons obtenu un résultat transitoire ou partiel. En

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A STUDY OF THE TITAN YELLOW DYE LAKE METHODS FOR THE ESTIMATION OF SERUM MAGNESIUM

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During a recent study made in this laboratory, pertaining to levels of magnesium in serum in chronic alcoholism,[9] various modifications of the titan yellow-magnesium dye lake method were studied and compared.[2, 4, 6-8]

In the standard methods, the following steps are common: precipitation of proteins, addition of titan yellow solution to protein-free filtrate, addition of color stabilizer, and, finally, alkalization of the mixture. Some methods also include addition of calcium to the standard solutions. A series of experiments was made, in which the different methods of each step were performed in order to compare them. In precipitating proteins, trichloracetic and tungstic acids were used, both on undiluted serum[7] and on serum diluted with distilled water.[6] Varied concentrations of different batches of titan yellow were used. Gum ghatti,[2, 6, 7] gum acacia, and polyvinyl alcohol[7] were used as color stabilizers. The effects of the concentration of sodium hydroxide and addition of calcium to standards[6, 8] were also observed. Basically, the method used was that of Neill and Neely[6] with the series of experimental modifications as outlined above.

RESULTS

1. *Protein precipitation.*
   (a) Precipitation of proteins from undiluted serum with both trichloracetic and tungstic acids.
   (b) Precipitation of proteins from serum diluted 1:6 with distilled water prior to the addition of the precipitants.

The results are best illustrated in tabular form (Table 1). From the table, it is apparent that protein-free filtrates prepared from undiluted serum contain less magnesium than those prepared from previously diluted serum. The results suggest that some of the "protein-bound" magnesium is carried down with the protein precipitate, and, further, that diluting serum liberates magnesium from the proteins, in such a manner that it is not carried down subsequently with the protein precipitate.

2. *Varying the concentration of titan yellow.* The concentrations of titan yellow used varied from 0.0075 per cent (w/v)[7] to 0.05 per cent (w/v).[2, 4] The only effect noted was that the color of the blank solution increased with the increase in concentration of titan yellow, making the setting of zero optical density difficult with some machines. Results obtained with all concentrations of titan yellow were identical. Some batches of titan yellow were observed to be unsuitable because of poor color yield with magnesium.

3. *Varied stabilizing agents.*
   (a) Gum ghatti,[2, 6, 7] using concentrations of 0.1 per cent (w/v), 0.5 per cent (w/v), and 2.0 per cent (w/v). No difference in the final result was observed.
   (b) Gum acacia, using the same concentrations as above, yielded results identical with those obtained with gum ghatti.
   (c) Polyvinyl alcohol.[7] Using this substance as a color-stabilizing agent, a much greater color intensity was produced, as illustrated graphically in Figure 1.

During the course of study, it was observed that when using serum from which the proteins had not been removed, the concentration of color was similar to that of a protein-free filtrate of the same serum to which polyvinyl alcohol had been added. On an average, results with serum containing proteins were 0.1 to 0.2 mg. per 100 ml.

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Mr. Spare is Chief Technician and Biochemist.

#4010

TABLE 1

COMPARATIVE RESULTS OF DETERMINATIONS OF MAGNESIUM WITH TWO PRECIPITANTS, AND UNDILUTED OR DILUTED SERUM

Number of Specimen	Method A-1*	Method A-2†	Method B-1‡	Method B-2§
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1	1.5	1.6	2.3	2.3
2	1.7	1.6	2.3	2.2
3	1.8	1.8	2.4	2.4
4	1.4	1.7	1.9	1.9
5	1.6	1.6	2.1	2.0
6	1.7	1.5	1.9	1.9
9	2.5	2.5	3.0	3.1
10	2.8	2.7	3.4	3.3

* Trichloracetic acid filtrate from undiluted serum.

† Tungstic acid filtrate from undiluted serum.

‡ Trichloracetic acid filtrate from previously diluted serum.

§ Tungstic acid filtrate from previously diluted serum.

of serum higher than with protein-free filtrates (Table 2). It was, therefore, apparent that serum proteins acted as color-intensifiers in the same way as polyvinyl alcohol. Having confirmed that serum proteins act as color intensifiers, experiments were conducted in order to elucidate the fraction of serum proteins possessing this property.

Bovine albumins and human albumins in concentrations up to 10 per cent (w/v) manifested no color intensification. Human globulins (Connaught Laboratories, gamma globulin solution) in 3 per cent (w/v) solution, however, when added to a previously protein-free filtrate, produced results identical with those produced by polyvinyl alcohol.

Starches (British drug houses) and dextrans (Abbott Laboratories), as color-intensifiers, failed to produce an effect.

It is interesting to compare the molecular weights of the different substances used in

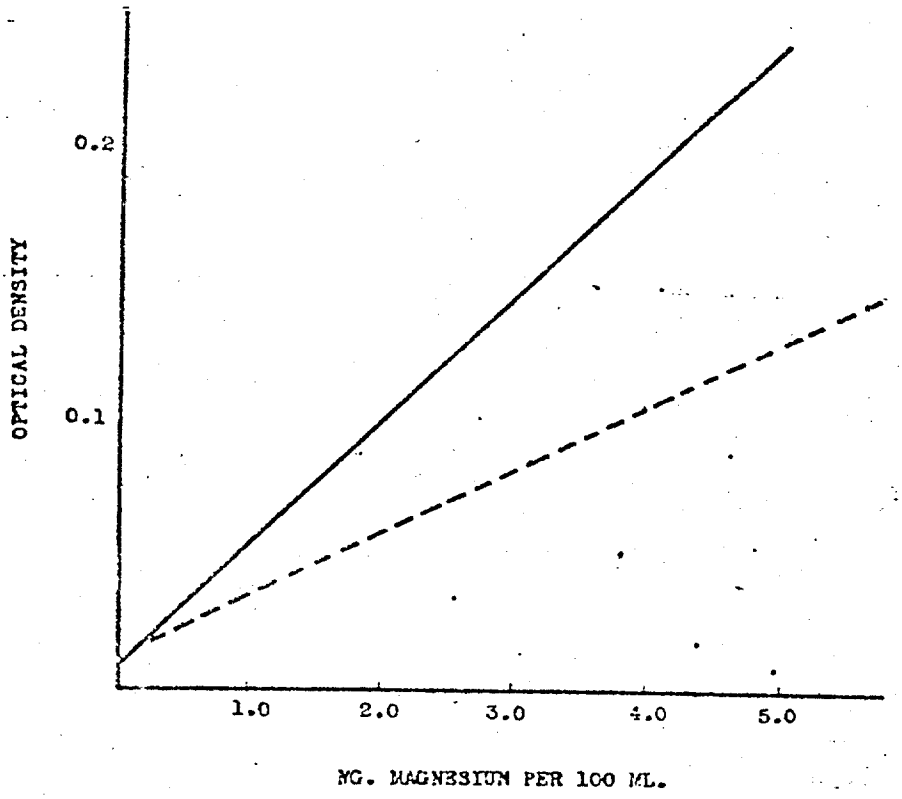

FIG. 1. Results with starches, dextrans, gums, and albumins (as color-stabilizing agents) are indicated by means of the *dotted line*; results with polyvinyl alcohol and human globulins are indicated by means of the *solid line*.

TABLE 2

COMPARATIVE RESULTS OF DETERMINATIONS OF MAGNESIUM IN SERUM AND IN PROTEIN-FREE FILTRATE OF SERUM

Number of Specimen	Method A*	Method B†
	mg./100 ml.	*mg./100 ml.*
1	2.4	2.6
2	2.4	2.5
3	2.6	2.8
4	2.2	2.3
5	2.1	2.0
6	2.4	2.4
7	2.6	2.7
8	3.3	3.6
9	2.7	3.0
10	3.0	3.1
11	3.4	3.6
12	2.5	2.6

* Protein-free filtrates prepared from previously diluted serum.

† Without removal of serum proteins.

TABLE 3

COMPARATIVE COLOR-INTENSIFYING EFFECTS OF VARIOUS SUBSTANCES

Substance	Molecular Weight	Color-Intensifying Effect
Gum acacia	1,200[10]	None
Gum ghatti	Probably similar to gum acacia	None
Soluble starches	60,000[1]	None
Dextrans	75,000[5]	None
Polyvinyl alcohol	200,000[3]	+++
Human albumins	69,000[4]	None
Human gamma globulins	300,000[4]	+++

the above experiments (Table 3). It will be noted that the substances which have a color-intensifying effect also have very high molecular weights.

4. *Alkalinization of the mixture.* The standard methods in the literature use concentrations of sodium hydroxide from 7.5 per cent (w/v) to 16 per cent (w/v). No effect on the final color was observed when using these concentrations.

5. *Addition of calcium ions to the standard solutions.* There is much disagreement in the literature as to whether or not calcium is a necessary additive to the standard solutions in estimation of magnesium. When using 0.02 mg. of calcium per milliliter of each standard solution, no effect was observed when compared with calcium-free standards.

DISCUSSION

On the basis of the experiments performed as described above, a new modification of the titan yellow method for serum magnesium estimations is proposed.

Serum proteins are not removed because, (1) they remain in solution during the addition of subsequent reagents; (2) all serum magnesium will remain in the reaction tube, inasmuch as no magnesium can be lost by precipitation with the proteins; and (3) the globulins act as color-intensifiers. Polyvinyl alcohol is used as the color-intensifier and stabilizer, which does not increase the color of a protein-containing fluid, but intensifies the color of protein-free standard solutions to an extent equal to that produced by serum globulins.

METHOD

All glassware must be chemically clean, and distilled water must be free from all traces of magnesium ions.

Reagents

1. *Stock standard magnesium solution.* Dissolve 8.458 Gm. of magnesium chloride ($MgCl_2 \cdot 6H_2O$) or 8.8178 Gm. of magnesium acetate ($Mg(CH_3COO)_2 \cdot 4H_2O$) in distilled water and make the volume up to 1 liter. This solution may be stored indefinitely in a polyethylene bottle.

2. *Working standard magnesium solution.* Dilute 1.0 ml. of the stock standard magnesium solution to 100 ml. with distilled water (1.0 ml. contains 0.005 mg.). This solution may be stored for at least a month in a polyethylene bottle.

3. *Polyvinyl alcohol, 0.1 per cent (w/v).* (K & K Laboratories, Inc., Long Island City, N. Y.). Dissolve 1.0 Gm. of polyvinyl alcohol in distilled water, using gentle heat. Make the volume up to 1 liter with distilled water. This solution is stable indefinitely in a polyethylene bottle.

4. *Stock 0.5 per cent titan yellow solution.*

Dissolve 0.5 Gm. of titan yellow in distilled water and make the volume up to 100 ml. This solution keeps indefinitely.

5. *Titan yellow solution, 0.01 per cent (w/v).* Prepare fresh each day by means of diluting 2.0 ml. of the above 0.5 per cent (w/v) titan yellow solution to 100 ml. with distilled water.

6. *Sodium hydroxide solution, 7.5 per cent (w/v).* Dissolve 15.0 Gm. of AnaLaR grade sodium hydroxide pellets in distilled water and make the volume up to 200 ml. Store in a well stoppered bottle.

In test tubes or colorimeter tubes place:

	Test ml.	Standard 1 ml.	Standard 2 ml.	Blank ml.
Distilled water	2.8	2.0	1.0	3.0
Serum	0.2	Nil	Nil	Nil
Standard magnesium solution (1)	Nil	1.0	2.0	Nil
Polyvinyl alcohol, 0.1 per cent (w/v)	0.5	0.5	0.5	0.5
Titan yellow, 0.01 per cent (w/v)	0.5	0.5	0.5	0.5
Sodium hydroxide, 7.5 per cent (w/v)	1.0	1.0	1.0	1.0

Mix after the addition of each reagent. After 5 min., read the optical densities of the test and standard solutions against the blank at a wave length setting of 540 mμ.

Calculations. Using standard 1:

$$\frac{\text{O.D. test}}{\text{O.D. standard 1}} \times 0.005 \times \frac{100}{0.2}$$

= mg. of magnesium/100 ml. of serum

$$\frac{\text{O.D. test}}{\text{O.D. standard 1}} \times 2.5$$

= mg. of magnesium/100 ml. of serum.

Using standard 2:

$$\frac{\text{O.D. test}}{\text{O.D. standard 2}} \times 0.01 \times \frac{100}{0.2}$$

= mg. of magnesium/100 ml. of serum

$$\frac{\text{O.D. test}}{\text{O.D. standard 2}} \times 5.0$$

= mg. of magnesium/100 ml. of serum.

In all experiments in this study and with the present method, 100 per cent recovery of added magnesium was obtained.

SUMMARY

Various modifications of the titan yellow dye lake method for the estimation of magnesium were investigated. It was observed that dilution of serum prior to the precipitation of proteins was necessary for complete recovery of "protein-bound" magnesium. Variations in the concentrations of titan yellow and sodium hydroxide had no effect on the final results. Addition of calcium ions to the standard solutions did not influence the color intensity. Polyvinyl alcohol and human gamma globulins have an equal color-intensifying effect, which is not produced by albumins, gums, starches, or dextrans. This property is possibly related to molecular size. A modification of the titan yellow-magnesium dye lake method is described, in which the color reaction is applied directly to serum, using polyvinyl alcohol as the color-stabilizing agent. The practical advantages of the method are the small amount of serum required, the enhancement of production of color, and the avoidance of loss of magnesium on protein precipitates.

SUMMARIO IN INTERLINGUA

Esseva investigate varie modificationes del methodo a lacca ex jalne Clayton e magnesium, usate in le estimation del contento de magnesium in specimens de sero. Esseva observate que le dilution del sero ante le precipitation de proteinas esseva necessari pro le complete inclusion, in le estimation final, del magnesium "ligate a proteina." Variationes in le concentration del jalne Clayton e del hydroxydo de natrium habeva nulle effecto super le resultatos final. Le addition de iones de calcium al solutiones standard exerceva nulle influentia super le intensitate del color. Alcohol polyvinylic e globulinas gamma human ha le mesme effecto intensificatori in le color. Iste effecto non es producite per albuminas, gummas, amylos o dextranos. Iste proprietate—le proprietate de intensificar le color—es possibilemente relationate al dimensiones del molecula. Es describite un modification del methodo a

lacca ex jalne Clayton e magnesium. In illo le chromo-reaction es applicate directemente al sero, con le uso de alcohol polyvinylic como agente de chromo-stabilisation. Le avantages practic de iste methodo es le micre quantitate de sero requirite, le meliorate production de color, e le elimination de omne perdita de magnesium in le precipitatos de proteina.

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Z. f. d. ges. exp. Med.
88:639-641, 1933.

Influence of Magnesium Sulfate on Urinary Excretion in Dogs

by

E.N. Speranskaja-Stepanowa

Received: Jan. 15, 1933.

Many investigators have studied the problem of the effect of various narcotics on renal activity (Kugel, Molitor and Pick, Fee, Stehle and Bourne, Epstein, Brings, Bonsmann and many others).

Since the use of magnesium sulfate for purposes of anesthesia has aroused considerable interest in recent years and since there are no data in the literature on the effect of this substance on urinary excretion, I have made a thorough study of the influence of magnesium on renal activity.

The experiments were carried out on 3 dogs with chronic fistulas of the ureters. Crystalline $MgSO_4$ was used in a 20% and 50% solution, the quantity ranging between 0.08 and 0.4 g per kg body weight. It should be noted that doses higher than 0.4 per kg body weight gave rise to a distinct reduction in the quantity of urine in some experiments. Most experiments were carried out with doses of 0.08 g of a 50% solution of $MgSO_4$ per kg body weight. If all necessary measures were adhered to no alterations were observed at the site of injection. In some dogs the injections of 50% solution were painful and the animals licked the skin at the puncture site.

Upon administration of 0.08 g per kg body weight a state of somnolence developed in the dogs some 20-30 min after the injection, which gradually intensified. Roughly one hour after the injection sleep was profound, the dog lay suspended in his support. Nevertheless he still reacted to hearing his name called, raised his eye lids and pricked up his ears.

Spontaneous urinary excretion was not influenced by magnesium sulfate. In order to increase the activity of the kidneys the organism was loaded with water by administering a certain quantity of warm water either by stomach tube or through the stomach fistula. In preliminary experiments (of several days' duration) the course of urinary excretion was established after water loading. Next the water was administered during magnesium narcosis. Gastric activity was not influenced by magnesium sulfate; the speed of transfer of the water into the duodenum did not change in comparison with the normal. In one series of experiments the substance under study was administered 15 to 30 min before the water intake. In another series the water was given 1 hour after the injection of magnesium, in other words when the narcotizing effect of the substance had already unfolded. In both series of experiments urinary excretion did not change compared to the normal (see Fig. 1).

The experimental results obtained apparently do not accord with those of Brings. This author observed a reduction in urinary excretion in the rabbit during $MgSO_4$ narcosis. It

should, however, be noted that this author administered considerably higher doses of magnesium than the quantities used by me. In some of my experiments, as has already been mentioned, doses higher than 0.4 g $MgSO_4$ per kg body weight gave rise to a distinct reduction in the urinary quantity. Brings has observed a difference in the effect of magnesium sulfate and magnesium chloride on urinary excretion: he observed a distinct reduction in diuresis upon administration of magnesium sulfate. He has concluded that this substance inhibits water excretion because it exercises a narcotizing effect on the midbrain. The introduction of chlorides, either separately or in combination with magnesium, as in $MgCl_2$, eliminates the inhibiting effect. However, Brings has disregarded the fact that when administering magnesium sulfate the quantity was almost twice as large as upon injection of magnesium chloride: magnesium sulfate was administered in a quantity of 1.3 g per kg body weight of the experimental animal (p. 365, experiment 4) and magnesium chloride in a quantity of 0.6 g (p. 367, experiment 2). In his control experiments with simultaneous administration of magnesium sulfate and chlorides the quantity of the former was equal to 0.9 g per kg (p. 370, experiment D). It will thus be easily understood that in this case no reduction is observed in urinary excretion. If we recalculate the quantities used by Brings to active magnesium ions, the ratio remains the same. In experiment 4 with magnesium sulfate 0.227 g Mg per kg body weight was

introduced if we use the magnesium sulfate formula $H_2O+MgSO_4$ and 0.126 g if we use the formula 7 $H_2O+MgSO_4$. In the experiments with magnesium chloride (6 $H_2O+MgCl_2$) 0.078 g Mg was introduced per kg body weight. Thus the quantity of Mg in the experiments with magnesium sulfate was roughly twice as large. In the control experiments with chlorides (experiment D) and magnesium sulfate (7 $H_2O+MgSO_4$) the dose of 0.095 g Mg per kg body weight was smaller than in the experiment with $MgSO_4$ alone. It is therefore possible that the difference observed by Brings in the effect on diuresis of the two salts is attributable merely to the quantity of magnesium administered.

Urinary excretion is strongly inhibited by morphine (Speranskaja-Stepanowa). Thus in some cases it would be very desirable to replace morphine with other narcotic agents. Of the agents already in use magnesium sulfate may be considered, since, as has been demonstrated by the experiments described above, it has no effect on urinary excretion in given quantities.

Conclusions

Sleep is induced in dogs by subcutaneous injection of 0.08 g magnesium sulfate per kg body weight. Urinary excretion is not influenced by this quantity.

Figure

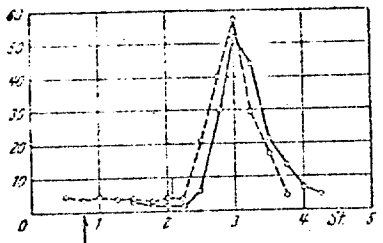

Abb. 1. Hund „Norka", Gewicht 6200 g. Beide Harnleiter zusammen ausgeführt. Abszissen-Zeit in Stunden, Ordinaten-Harnmenge in Kubikzentimeter. ——— Versuch vom 7. 12. 31 (ununterbrochene Linie). Gang der normalen Harnausscheidung nach der Verabreichung von 300 ccm Wasser (durch die Magenfistel); die Zeit der Verabreichung durch eine senkrechte Linie vermerkt. — — — Versuch vom 8. 12. 31 (unterbrochene Linie). Gang der Harnausscheidung nach der Verabreichung von 300 ccm Wasser während der Magnesiumnarkose. Der Augenblick der Injektion von schwefelsaurem Magnesium — 1 ccm einer 50%igen Lösung — ist durch einen Pfeil angegeben (0,08 g $MgSO_4$ pro Kilo Körpergewicht).

1. Dog "Norka", weight 6200 g. Both ureters evacuated simultaneously. Abscissa: time in hours. Ordinate: urine quantity in cc. Solid line: preliminary experiment Dec. 7, 1931. Course of normal urinary excretion after administration of 300 cc water (through stomach fistula); time of administration marked by vertical line. Broken line: experiment on Dec. 8, 1931. Course of urinary excretion after administration of 300 cc water during magnesium narcosis. The moment of injection of 1 cc of a 50% solution of magnesium sulfate indicated by arrow (0.08 g $MgSO_4$ per kg body weight).

Bibliography

See p. 641.

(Aus dem Pharmakologischen Laboratorium des staatlichen Instituts für experimentelle Medizin in Leningrad.)

Über den Einfluß von schwefelsaurem Magnesium auf die Harnausscheidung bei Hunden.

Von

E. N. Speranskaja-Stepanowa.

Mit 1 Textabbildung.

(Eingegangen am 15. Januar 1933.)

Die Frage über die Beeinflussung der Nierentätigkeit durch verschiedene Narkotica ist von vielen Forschern untersucht worden (*Kugel, Filbor* und *Pick, Fee, Stehle* und *Bourne, Epstein, Brings, Bonsmann* und vielen anderen).

Da die Frage der Anwendung von schwefelsaurem Magnesium für Zwecke der Narkose in den letzten Jahren ein sehr reges Interesse erweckt hat, in der Literatur keine Angaben über die Wirkung dieses Stoffes auf die Harnausscheidung vorliegen, habe ich den Einfluß von Magnesium auf die Nierentätigkeit eingehend untersucht.

Die Versuche sind an *drei* Hunden mit chronischen Harnleiterfisteln ausgeführt. $MgSO_4$ crystallisat. wurde in 20- und 50%iger Lösungen angewandt, wobei die Dosen von *0,08–0,1 g* pro Kilogramm Körpergewicht schwankte. Es muß bemerkt werden, daß größere Dosen als 0,1 pro Kilogramm Körpergewicht in einigen Versuchen eine deutliche Verminderung der Harnmenge hervorriefen. Die Mehrzahl der Versuche sind mit Dosen von 0,08 g $MgSO_4$ einer 50%igen Lösung pro Kilogramm Tiergewicht ausgeführt. Wenn alle nötigen Maßnahmen vorgenommen wurden, so zeigten sich an den Injektionsstellen keine Veränderungen beobachten. Bei einigen Versuchen waren die Injektionen von 50%iger Lösung schmerzhaft, die Tiere beleckten oft Haut an der Einstichstelle.

Bei der Verabreichung von 0,08 g pro Kilogramm Körpergewicht tritt beim Hunde etwa 20–30 Minuten nach der Injektion ein Zustand von Schläfrigkeit auf, die allmählich immer stärker und stärker wird. Ungefähr 1 Stunde nach der Injektion ist der Schlaf bereits tief, der Hund liegt im Gestell; doch ist die Reaktion auf den Namensruf erhalten — das Tier hebt die Augenlider und spitzt die Ohren.

Die spontane Harnausscheidung wird durch schwefelsaures Magnesium nicht beeinflußt. Um die Tätigkeit der Nieren zu verstärken, wurde die Belastung des Organismus mit Wasser vorgenommen, wobei die berechnete bestimmte Menge warmen Wassers entweder mit Hilfe der Magensonde oder durch die Magenfistel verabreicht wurde. In Vorversuchen

wurde (im Laufe von mehreren Tagen) der Gang der Harnausscheidung nach der Wasserbelastung festgestellt. Darauf wurde das Wasser während der Magnesiumnarkose verabreicht. Die Magentätigkeit wird durch schwefelsaures Magnesium nicht beeinträchtigt — die Geschwindigkeit des Überganges des Wassers ins Duodenum ist gegen die Norm nicht verändert. In einer Versuchsreihe wurde der untersuchte Stoff 15 bis 30 Minuten vor der Wasserzufuhr verabreicht; in einer anderen Versuchsreihe wurde das Wasser 1 Stunde nach der Injektion des Magnesiums verabreicht, also zur Zeit, wenn die narkotisierende Wirkung dieses Stoffes bereits eingetreten war. In beiden Versuchsreihen blieb die Harnausscheidung gegen die Norm unverändert (vgl. Abb. 1).

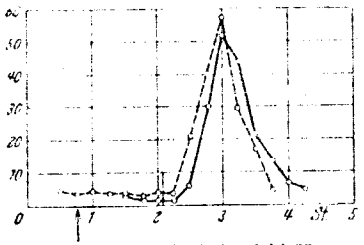

Abb. 1. Hund „Norka", Gewicht 6200 g. Beide Harnleiter zusammen ausgeführt. Abszissen-Zeit in Stunden, Ordinaten-Harnmenge in Kubikzentimeter. ——— Versuch vom 7. 12. 31 (ununterbrochene Linie). Gang der normalen Harnausscheidung nach der Verabreichung von 300 ccm Wasser (durch die Magenfistel); die Zeit der Verabreichung durch eine senkrechte Linie vermerkt. — — — Versuch vom 8. 12. 31 (unterbrochene Linie). Gang der Harnausscheidung nach der Verabreichung von 300 ccm Wasser während der Magnesiumnarkose. Der Augenblick der Injektion von schwefelsaurem Magnesium — 1 ccm einer 50%igen Lösung — ist durch einen Pfeil angegeben (0,08 g $MgSO_4$ pro Kilo Körpergewicht).

Die erhaltenen Versuchsergebnisse stimmen, wie es scheinen könnte, mit den Angaben von *Brings* nicht überein: der genannte Autor hat bei Kaninchen eine Verringerung der Harnausscheidung während der $MgSO_4$-Narkose beobachtet. Es muß aber bemerkt werden, daß dieser Autor bedeutend größere Dosen Magnesium verabreicht hat, als die von mir angewandten Mengen. In einigen von meinen Versuchen, wie es schon erwähnt war, riefen die größeren Dosen als 0,4 g $MgSO_4$ pro Kilogramm Körpergewicht eine deutliche Verminderung der Harnmenge hervor. *Brings* hat einen Unterschied in der Beeinflussung der Harnausscheidung durch schwefelsaures Magnesium und Chlormagnesium beobachtet; er hat bei der Verabreichung von schwefelsaurem Magnesium eine bedeutende Verringerung der Diurese festgestellt; er ist zum Schlusse gekommen, daß dieser Stoff die Wasserausscheidung deshalb hemmt, weil er eine narkotisierende Wirkung auf das Mittelhirn ausübt. Die Einführung von Chloriden, sei es getrennt oder mit Magnesium verbunden, wie $MgCl_2$ hebt die hemmende Wirkung auf. Doch hat *Brings* außer acht gelassen, daß bei der Verabreichung von schwefelsaurem Magnesium die Menge fast doppelt so groß war als bei der Injektion von Chlormagnesium: schwefelsaures Magnesium wurde in einer Menge von 1,3 g pro Kilogramm Körpergewicht des Versuchstieres (S. 365, Versuch Nr. 4) und Chlormagnesium in einer Menge von 0,6 g appliziert (S. 367, Versuch Nr. 2). In seinen Kontrollversuchen bei der gleichzeitigen Verabreichung von schwefelsaurem Magnesium und von

Chloriden war die Menge des ersteren gleich 0,9 g pro Kilogramm (S. 370, Versuch D); es ist deshalb gut verständlich, daß in diesem Falle keine Verringerung der Harnausscheidung beobachtet ist. Wenn wir die von *Brings* angewandten Mengen auf die aktiven Magnesiumionen umrechnen, bleibt das Verhältnis gleich. Im Versuche Nr. 4 mit schwefelsaurem Magnesium war Mg 0,227 g pro Kilogramm Körpergewicht eingeführt, wenn man die Formel schwefelsaures Magnesium $H_2O + MgSO_4$ und 0,126 g bei der Formel $7\ H_2O + MgSO_4$ berechnet. In den Versuchen mit chlorsaurem Magnesium ($6\ H_2O + MgCl_2$) auf 1 Kilo Gewicht wurde 0,078 g Mg eingeführt. Auf diese Weise war die Quantität des Mg in den Versuchen mit schwefelsaurem Magnesium ungefähr zweimal größer. In dem Kontrollversuche mit Chloriden (Versuch D) und schwefelsaurem Magnesium ($7\ H_2O + MgSO_4$) war Mg 0,095 g auf 1 Kilo Gewicht die Dose kleiner als in dem Versuche mit $MgSO_4$ allein. Es ist deshalb möglich, daß der von *Brings* beobachtete Unterschied in der Beeinflussung der Diurese durch die beiden Salze lediglich von der Menge des verabreichten Magnesiums zurückgeführt werden kann.

Durch Morphium wird die Harnausscheidung sehr stark aufgehalten (*Speranskaja-Stepanowa*). Deshalb könnte in einigen Fällen eine Ersetzung von Morphium durch andere Narkotica sehr wünschenswert sein. Aus den Mitteln, die bereits angewandt werden, kann man auf schwefelsaures Magnesium hinweisen, das, wie durch die beschriebenen Versuche gezeigt ist, in bestimmten Mengen die Harnausscheidung unbeeinträchtigt läßt.

Schlußfolgerung.

Durch subcutane Injektionen von schwefelsaurem Magnesium in einer Menge von 0,08 g pro Kilogramm Körpergewicht wird bei Hunden Schlaf erzeugt. Die Harnausscheidung wird durch diese Menge nicht beeinflußt.

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MEASUREMENTS OF SERUM CALCIUM AND MAGNESIUM BY ATOMIC ABSORPTION SPECTROMETRY

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In 1860, Kirchoff and Bunsen[15] observed that the wave lengths of the dark Fraunhofer absorption lines in the solar spectrum coincided with the wave lengths of elemental lines in various emission spectrums. Based upon the assumption that atoms absorb light at the same wave lengths as they emit light, Kirchoff and Bunsen[15] deduced the presence of several elements in the solar atmosphere.

In 1955, Walsh[24] showed that the phenomenon of atomic absorption could serve as a spectrochemical basis for the quantitative determination of metals. He demonstrated that measurements of metals by atomic absorption spectrometry are more sensitive than measurements by flame emission spectrometry, and less subject to interference from other elements.[24]

Within the past decade more than 100 papers have been published which describe analytic applications of atomic absorption spectrometry. The recent literature on atomic absorption has been comprehensively reviewed by Allan,[3] David,[8] Elwell and Gidley,[10] Robinson,[21] and Willis.[30] The theoretic and instrumental factors which influence quantitative determinations of metals by atomic absorption spectrometry have been summarized by Allan,[2] Fuwa and Vallee,[11] Malmstadt and Chambers,[17] Menzies,[18] Millazo,[19] and Russell and associates.[22]

An atomic absorption method for the determination of calcium in biologic materials was reported in 1960 by Willis,[26, 27, 29] and has been modified by Newbrun[20] and by Zettner and Seligson.[31] Use of atomic absorption spectrometry for the determination of magnesium in biologic materials was reported in 1959 by Willis,[25, 28, 29] and has been modified by Dawson and Heaton[9] and by Horn and Latner.[13] A further modification of these atomic absorption procedures, which enables precise determinations of calcium and magnesium to be performed upon a single dilution of a protein-free filtrate of serum, is described in this paper.

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METHOD

Principle

The atomic absorption spectrometer employed in our laboratory is illustrated schematically in Figure 1. Aspiration of a protein-free filtrate of serum into the burner produces thermal molecular dissociation and dispersion of calcium and magnesium atoms throughout the flame. Small proportions of these atoms become excited to emit light, but the overwhelming majorities of the atoms remain in the ground state and are capable of absorbing discrete wave lengths of incident light. These specific wave lengths are provided by a lamp with a hollow cathode constructed of an alloy containing both calcium and magnesium. The beam of light is passed through the flame several times, and then is focused upon the entrance slit of a diffraction grating monochromator. The absorptions of light at the specific wave lengths are proportional to the concentrations of calcium and magnesium in the sample.

The multi-passing optical system increases the optical path length within the flame, and thereby enhances the sensitivity of the atomic absorption technic. The system also minimizes fluctuations in atomic absorption which may result from variable distribution of ground-state atoms within the flame.[13, 25] The abundance of atoms in the ground state in comparison with those in the excited state accounts for the greater sensitivity of atomic absorption spec-

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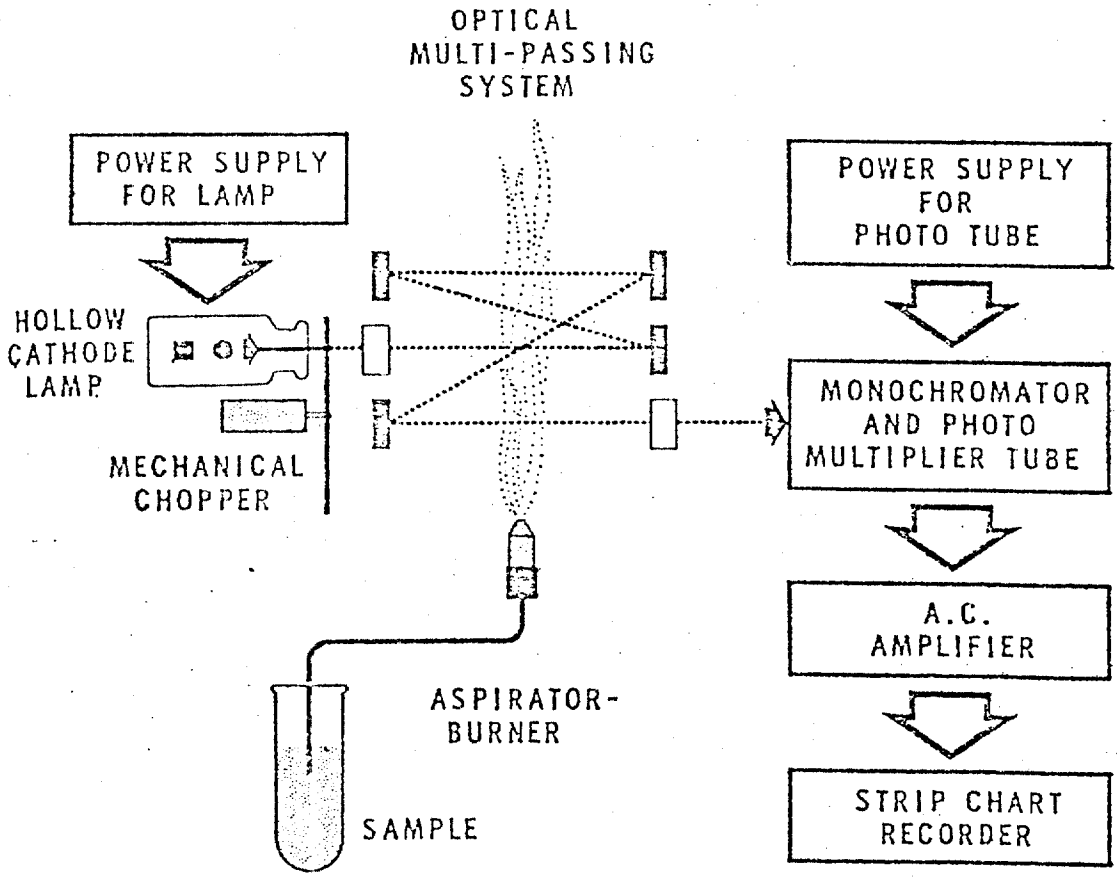

FIG. 1. Schematic diagram of the atomic absorption spectrometer

trometry relative to flame emission spectrometry.

Light emitted from the flame constitutes a potential source of error in atomic absorption spectrometry, as the light energy which strikes the photomultiplier tube represents the net balance of emission and absorption. This source of error is avoided by modulating the incident light beam with a mechanical chopper, and tuning the photomultiplier detector circuit to the same frequency of modulation. Under these conditions, the detector circuit responds only to the pulsed signal from the light beam, and does not respond to the continuous signal produced by light emission from the flame.[31] The alternating current from the photomultiplier detector circuit is amplified and recorded.

Reagents

1. *Strontium chloride solution in 0.2 N hydrochloric acid.* Into a 1-liter volumetric flask are transferred 15.2 Gm. of strontium chloride ($SrCl_2 \cdot 6H_2O$). Concentrated hydrochloric acid, 17.5 ml., is added to the flask. The contents are dissolved in distilled water and diluted to the mark. Each lot of strontium chloride reagent should be tested to verify that it is not contaminated with calcium or magnesium.

2. *Sodium chloride solution* (70 mEq. per liter). Into a 1-liter volumetric flask are transferred 4.09 Gm. of reagent grade sodium chloride. The contents of the flask are dissolved in distilled water and diluted to the calibration mark.

3. *Trichloracetic acid solution,* 10 per cent (w/v).

4. *Trichloracetic acid solution,* 22.5 per cent (w/v).

5. *Calcium stock standard solution* (10 mg. of calcium per 100 ml.). Reagent grade calcium carbonate is dried at 120 C. for 4 hr. and then cooled in a desiccator. Into a 1-liter

volumetric flask is placed 0.2498 Gm. of calcium carbonate. Ten milliliters of 1.0 N hydrochloric acid are added, and after the calcium carbonate has dissolved, the contents of the flask are diluted with water and mixed. With the temperature at 25 C., the volume is adjusted to the calibration mark.

6. *Calcium working standard solutions.* Into 50-ml. volumetric flasks are transferred 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ml. of calcium stock standard solution. Twenty-five milliliters of strontium chloride solution, 5 ml. of sodium chloride solution, and 10 ml. of 22.5 per cent trichloracetic acid solution are added to each flask, and the contents are diluted to the mark with distilled water. These standard solutions are equivalent to 0, 2, 4, 6, 8, 10, 12, and 14 mg. of calcium per 100 ml. of serum.

7. *Magnesium stock standard solution* (2 mg. of magnesium per 100 ml.). Into a 1-liter volumetric flask is placed 0.2027 Gm. of magnesium sulfate ($MgSO_4 \cdot 7H_2O$). The contents of the flask are diluted with water and mixed. With the temperature at 25 C., the volume is adjusted to the mark.

8. *Magnesium working standard solutions.* Into 50-ml. volumetric flasks are transferred 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ml. of magnesium stock standard solution. Twenty-five milliliters of strontium chloride solution, 5 ml. of sodium chloride solution, and 10 ml. of 22.5 per cent trichloracetic acid solution are added to each flask, and the contents are diluted to the mark with distilled water. These standard solutions are equivalent to 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, and 2.8 mg. of magnesium per 100 ml. of serum.

Atomic Absorption Apparatus

A Jarrell-Ash atomic absorption apparatus (model 82-362) is employed with the following modifications: (1) A Westinghouse hollow cathode discharge lamp (model WL-22604) is used for measurements of both calcium and magnesium. (2) A Zeiss hydrogen-air burner (model 50-56-23) is mounted at the midpoint of the optical path with the tip of the burner 14 cm. from the top of the optical bench. The cannula of the burner is attached to a 6-cm. length of polyethylene tubing, so that the sample may be aspirated from a test tube. Polyethylene tubing with an inside diameter of 0.030 in. is satisfactory (Clay-Adams tubing PE 60). (3) The output of the photomultiplier tube detector circuit is recorded by means of a 10-mv. strip-chart recorder (Honeywell Regulator Co., model E15-I).

Procedure

Preparation of sample. Into a 12-ml. centrifuge tube are transferred 0.5 ml. of serum and 4.5 ml. of 10 per cent trichloracetic acid. The contents of the tube are mixed with a Vortex rotary mixer and allowed to stand for 10 min. The tubes are centrifuged at 2500 r.p.m. for 15 min. Three milliliters of the clear supernatant are transferred to a test tube, 3 ml. of strontium chloride solution are added, and the contents of the test tube are mixed with a Vortex mixer.

Adjustment of the atomic absorption apparatus. The power supply to the calcium-magnesium hollow cathode lamp is adjusted to 20 ma. The beam of light from the hollow cathode lamp is focused upon the monochromator entrance slit. The monochromator is adjusted to measure the absorption at the wave lengths specified for calcium or magnesium in Table 1. The air regulator valve is opened first, followed by the hydrogen regulator valve, and the burner is ignited. The gas regulator valves are adjusted to provide the gas pressures specified in Table 1 for determinations of calcium or magnesium.

An opaque card is placed in front of the monochromator entrance slit and the recorder is adjusted to 100 per cent absorption. The card is removed and the recorder is adjusted to 0 per cent absorption while water is aspirated through the burner. Fluctuations in the baseline are minimized by use of the damping adjustment. Stability of the burner and the optical and electronic systems is verified by monitoring the baseline while water is aspirated through the burner.

Measurements of atomic absorption. The polyethylene tubing connected to the burner is inserted into a test tube which contains one of the standards or samples. The ab-

TABLE 1

OPERATING CONDITIONS FOR MEASUREMENTS OF SERUM CALCIUM AND MAGNESIUM BY ATOMIC ABSORPTION SPECTROMETRY

Calcium	
Sample aspiration rate	5.2 ml. per min.
Hydrogen pressure	15 lb. per sq. in.
Hydrogen flow rate	0.5 liters per min.
Air pressure	15 lb. per sq. in.
Lamp current	20 ma.
Absorption wave length	4227 Å
Entrance and exit slits	100 μ
Magnesium	
Sample aspiration rate	3.0 ml. per min.
Hydrogen pressure	5 lb. per sq. in.
Hydrogen flow rate	0.3 liters per min.
Air pressure	8 lb. per sq. in.
Lamp current	20 ma.
Absorption wave length	2852 Å
Entrance and exit slits	100 μ

sorption reading does not become stable until the sample has been aspirated into the flame for 2 to 3 sec. The atomic absorption of the sample is then recorded for 5 sec. Immediately after the aspiration of each sample, water is aspirated through the burner until the absorption reading has returned to the baseline. All measurements are performed in duplicate, and standard solutions of calcium or magnesium are analyzed with each group of samples.

At the conclusion of the analyses, water is aspirated through the burner for 5 min. The hydrogen regulator valve is closed, and the burner is dried by passing air through it for 1 to 2 min.

Calculations. Measurements of per cent absorption (per cent A) are converted into absorbance (O.D.) units by the equation:

$$\text{Absorbance (O.D.)} = 2 - \log_{10} (100 - \text{per cent A})$$

Calibration curves are used in computing the concentrations of calcium and magnesium in the samples of serum.

RESULTS

Instrumental Parameters

The absorption line of calcium at 4227 Å and the absorption line of magnesium at 2852 Å were used throughout these investigations. At these wave lengths, the optimal discharge current for the hollow cathode lamp was found to be 20 ma. Measurements of calcium and magnesium were performed with monochromator entrance and exit slits of 25-, 40-, 50-, and 100-μ widths. The greatest sensitivities were achieved with slit widths of 100 μ.

Comparisons were made of measurements of calcium and magnesium with fuel mixtures of hydrogen and air, acetylene and air, and acetylene and oxygen. The sensitivities obtained with a hydrogen-air flame were greater than those obtained with the other fuel mixtures. Moreover, the hydrogen-air flame provided the most stable baseline and the least visual and auditory irritation.

The Jarrell-Ash atomic absorption spectrometer is manufactured with a burner manifold on which 3 Beckman hydrogen-air burners are mounted. The reproducibility of measurements of calcium and magnesium with the Beckman burners was found to be less than with a single Zeiss burner (Table 2). With use of the 3 Beckman burners, the coefficients of variation of 10 replicate measurements of serum calcium and magnesium were 3.8 and 5.1 per cent. When a single Zeiss burner was substituted for the Beckman burners, the coefficients of variation of 10 replicate measurements of serum calcium and magnesium were 1.1 and 1.6 per cent.

As shown in Figure 2, calibration curves with the Beckman burners and the Zeiss burner had similar configurations. As the slopes of the calibration curves were influenced by small fluctuations in gas pressure and in monochromator adjustment, it was necessary to prepare a calibration curve with each group of analyses.

Sources of Interference

Our observations regarding the sources of interference in measurements of serum calcium and magnesium, as summarized in Table 3, confirmed the findings of other

TABLE 2

PRECISION OF MEASUREMENTS OF SERUM CALCIUM AND MAGNESIUM BY ATOMIC ABSORPTION SPECTROMETRY

	Beckman Burners		Zeiss Burner	
	mg./100 ml.	*per cent*	*mg./100 ml.*	*per cent*
Calcium				
Mean concentration	10.0		10.0	
Range	9.3 to 10.7		9.8 to 10.2	
Standard deviation	±0.38		±0.11	
Coefficient of variation		3.8		1.1
Magnesium				
Mean concentration	2.0		2.0	
Range	1.81 to 2.18		1.94 to 2.06	
Standard deviation	±0.10		±0.03	
Coefficient of variation		5.1		1.6

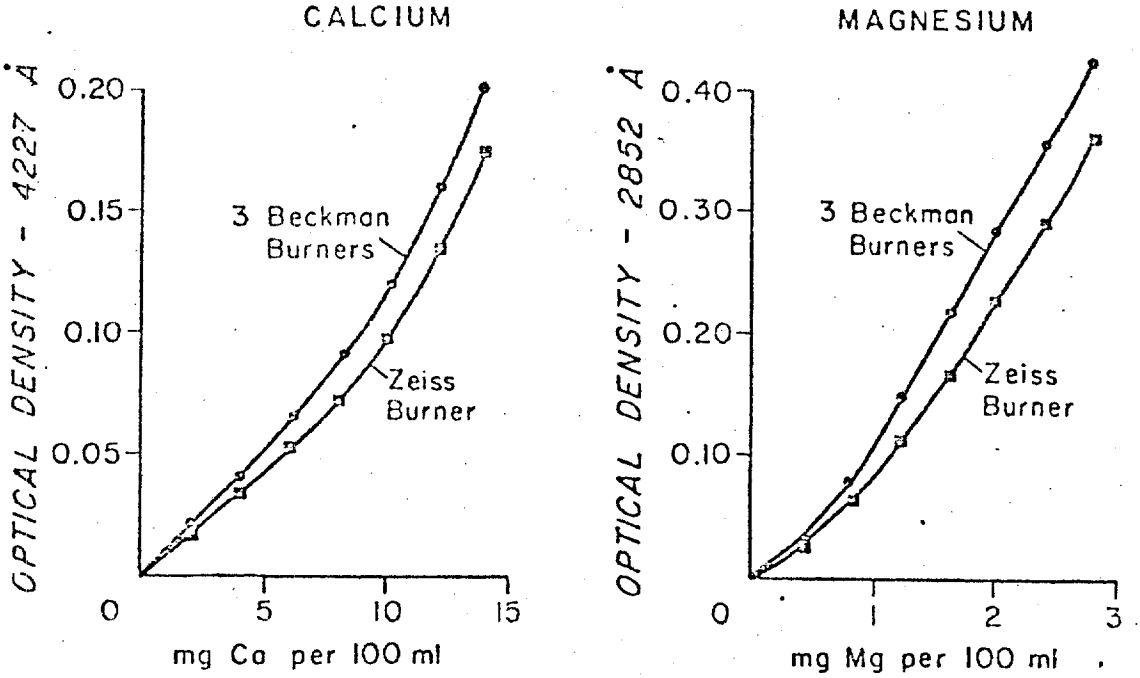

FIG. 2. Calibration curves for measurements of serum calcium and magnesium. The following gas pressures were used: (1) serum calcium with Beckman burners: 24 p.s.i. H_2, 25 p.s.i. air; (2) serum calcium with Zeiss burner: 15 p.s.i. H_2, 15 p.s.i. air; (3) serum magnesium with Beckman burners: 10 p.s.i. H_2, 10 p.s.i. air; (4) serum magnesium with Zeiss burner: 5 p.s.i. H_2, 8 p.s.i. air.

workers.[1, 7, 9, 13, 16, 23, 29, 31] Phosphate and sulfate produced diminutions in the atomic absorption of calcium owing to the formation of calcium salts which are resistant to thermal molecular dissociation.[8, 12] Interference from sulfate and phosphate may be prevented by additions of EDTA (ethylenediamine tetraacetic acid), strontium, or lanthanum.[5, 24, 26] EDTA prevents interference by binding calcium and thus avoiding the combination of calcium with sulfate and phosphate.[20] Strontium and lanthanum prevent interference by uniting preferentially with the sulfate and phosphate ions.[29] Strontium and lanthanum chlorides were added to serum filtrates and to standard solutions in final concentrations of 2,500, 5,000, and 10,000 p.p.m. of Sr or La. Under the condi-

TABLE 3

INTERFERENCES IN MEASUREMENTS OF SERUM CALCIUM AND MAGNESIUM BY ATOMIC ABSORPTION SPECTROMETRY

Source of Interference	Compensation for Interference
SO_4 and PO_4 interference in determination of Ca	Addition of EDTA, Sr, or La to samples and standards
Na interference in determination of Ca	Addition of Na to standards
Protein interference in determination of Ca and Mg	Protein precipitation with trichloracetic acid, or addition of protein to standards

TABLE 4

MEASUREMENTS OF RECOVERY OF CALCIUM AND MAGNESIUM ADDED TO SERUM

	per cent
Recovery of calcium added to 10 serums in concentration of 4 mg. per 100 ml.	
Mean recovery	100
Range	94 to 105
Coefficient of variation	3.3
Recovery of magnesium added to 8 serums in concentration of 0.8 mg. per 100 ml.	
Mean recovery	102
Range	96 to 107
Coefficient of variation	4.3

tions described for the procedure, additions of strontium or lanthanum in final concentrations of 2,500 p.p.m. prevented interference from sulfate and phosphate throughout the ranges of concentrations encountered in pathologic serums. Strontium was found to have the special advantage that the red color of its emission spectrum facilitated visual monitoring of the performance of the burner.

Under operating conditions, serum sodium produced a mean enhancement of 3 per cent in the atomic absorption of serum calcium. This source of error was avoided by the addition of sodium to the standard samples in a concentration equivalent to 140 mEq. per liter of serum. Sodium was not found to have a significant effect upon the atomic absorption of magnesium. Additions of potassium to the standard solutions in concentrations of 5 and 10 mEq. per liter did not influence the atomic absorption of calcium or magnesium. Additions of magnesium to the calcium standards in concentrations of 2 and 4 mg. per 100 ml., and additions of calcium to the magnesium standards in concentrations of 10 and 20 mg. per 100 ml., were likewise without effect.

Serum proteins produced inhibition in the atomic absorption of calcium as well as magnesium. To avoid protein interference, Newbrun[30] recommended the analysis of protein-free filtrates, whereas Zettner and Seligson[31] recommended the preparation of calcium-free serum proteins by ion exchange and dialysis, and addition of the decalcified proteins to the standard solutions in a concentration of 6.5 Gm. of protein per 100 ml. In our experience, preparation of trichloracetic acid filtrates of serum proved to be the most practical means of avoiding protein interference.

Recovery Studies

Measurements of the recovery of calcium and magnesium added to serum are listed in Table 4. Calcium stock standard solution was added to 10 samples of serum in order to increase the concentrations of calcium by 4 mg. per 100 ml. Magnesium stock standard solution was added to 8 samples of serum in order to increase the concentrations of magnesium by 0.8 mg. per 100 ml. As listed in Table 4, the recovery of calcium averaged 100 per cent with a range from 94 to 105 per cent. The recovery of magnesium averaged 102 per cent, with a range from 96 to 107 per cent.

Comparisons with Other Methods

In Figure 3, determinations of serum calcium by atomic absorption spectrometry are contrasted with measurements by the fluorometric EDTA procedure of Kepner and Hercules.[13] In analyses of 50 serums using the Beckman burners, the slope of the re-

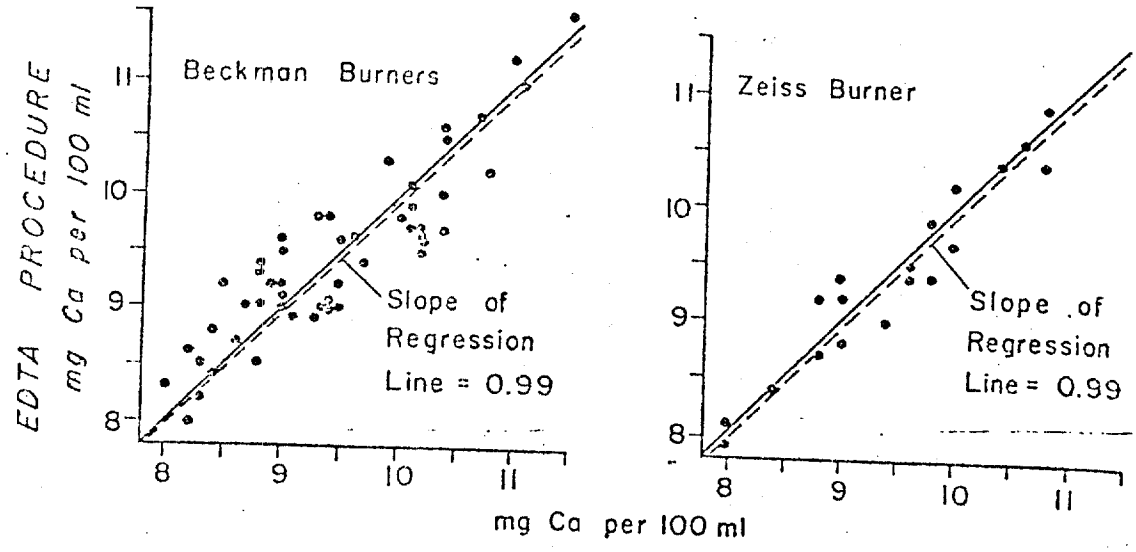

FIG. 3. Correlation of calcium measurements by atomic absorption and EDTA procedures

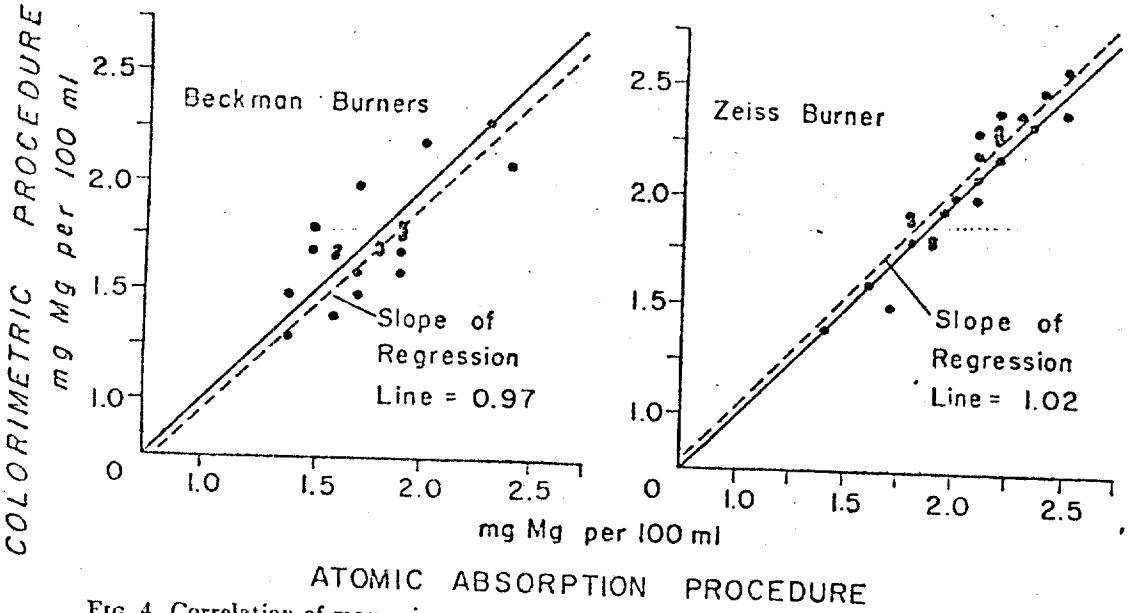

FIG. 4. Correlation of magnesium measurements by atomic absorption and colorimetric procedures

gression line was 0.99 and the standard error of estimate was ±0.39 mg. of calcium per 100 ml. In analyses of 19 serums using the Zeiss burner, the slope of the regression line was 0.99 and the standard error of estimate was ±0.23 mg. of calcium per 100 ml.

Measurements of serum magnesium by atomic absorption spectrometry and by the colorimetric procedure of Bohuon[4] are compared in Figure 4. In analyses of 20 serums using the Beckman burners, the slope of the regression line was 0.97, and the standard error of estimate was ±0.29 mg. of magnesium per 100 ml. In analyses of 24 serums using the Zeiss burner, the slope of the regression line was 1.02, and the standard

error of estimate was ±0.09 mg. of magnesium per 100 ml.

Twenty-milliliter samples of serum were obtained from 10 apparently normal subjects. The concentrations of calcium and magnesium were determined in these samples by the Clark-Collip[6] and Briggs[5] procedures. With each of these samples, the means of replicate analyses of calcium and magnesium by atomic absorption spectrometry agreed with the means of replicate analyses by the reference procedures within ±2 per cent.

SUMMARY

A procedure for atomic absorption spectrometry is described which enables determinations of calcium and magnesium to be performed upon a single dilution of a protein-free filtrate of serum. The advantages of the procedure include rapidity and sensitivity.

Interferences in measurements of serum calcium owing to sulfate and phosphate are avoided by additions of strontium chloride to the samples and standards. Interference of sodium in determinations of serum calcium is compensated for by additions of sodium chloride to the standard solutions. Interference of proteins in measurements of serum calcium and magnesium is eliminated by precipitation of the proteins with trichloracetic acid.

The standard deviations of replicate determinations of serum calcium and magnesium by the atomic absorption procedure are ±0.11 and ±0.03 mg. per 100 ml., respectively. The recoveries of calcium and magnesium added to serums average 100 ± 3.3 and 102 ± 4.3 per cent. In comparisons with reference chemical procedures, the standard error of estimate of serum calcium is ±0.23 mg. per 100 ml., and the standard error of estimate of serum magnesium is ±0.09 mg. per 100 ml.

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shutter closed. With the shutter open, the 6 mEq. per l. standard was set to read 100 per cent transmittance by adjusting the slit.

Colorimetric method. The procedure of Garner[2] was used with the inclusion of the hydroxylamine modification suggested by Kunkel and his associates.[4] Optical density readings were taken on the Coleman Junior and also the Beckman DU spectrophotometers at a wavelength of 550 mμ, with comparable results. Standards used in this method were the 0, 2, 4, and 6 mEq. per l. of stock standards treated in the same manner as the serum.

Stock standards. Solutions were prepared to contain 0, 1, 2, 3, 4, and 6 mEq. per l. of magnesium, by dissolving pure magnesium metal in hydrochloric acid. Each stock standard also contained 140 mEq. per l. of sodium, 5 mEq. per l. of calcium, 4 mEq. per l. of potassium, and 1 mM per l. of phosphorus as phosphate.

RESULTS

Typical readings for the standard curve for magnesium at 285.2 mμ are listed in Table 1. Although the curve is not linear, as has been reported for the curve taken at 371 mμ, the 285.2 mμ wavelength was preferred because of better selectivity. Even though standard curves were linear and reproducible at the 371 mμ wavelength, the results on samples of serum were too erratic for acceptance. Teloh[5] has adequately described the great interferences encountered at 371 mμ.

Using the method described, interference was investigated by adding various substances to pooled serum and determining the extent of alteration of the apparent concentration of magnesium. The pooled serum, without any added substance, was found to contain 1.67 mEq. per l. Increasing the sodium of one portion of the pooled serum from 129 to 164 mEq. per l. resulted in an apparent concentration of magnesium of 1.77 mEq. per l. Increasing the potassium in another portion of the pooled serum from 3.9 to 6.9 mEq. per l. resulted in an apparent concentration of magnesium of 1.77 mEq. per l. Similarly, altering the calcium from 4.5 to 7.5 mEq. per l. resulted in an apparent concentration of magnesium of 1.67, and changing the phosphorus (as phosphate) from 1.1 to 2.1 mM per l. in another portion of the pooled serum resulted in an apparent concentration of magnesium of 1.75 mEq. per l. The slight difference in the concentration of magnesium produced by the various substances added are within the range of experimental error. Interferences from these substances in the ranges of concentration studied may therefore be considered negligible.

The mean and standard deviation of 10 replicate analyses on serum that contained magnesium at 3 levels of concentration and the average recovery of magnesium calcu-

TABLE 1

STANDARD CURVE

Magnesium Concentration of Standard	Luminosity Reading
mEq./l.	
6.0	100
4.0	93
3.0	88
2.0	81
1.0	74
0.0	64

TABLE 2

COMPARISON OF THE TWO METHODS FOR ESTIMATION OF MAGNESIUM: THEIR ABSOLUTE VALUES, RECOVERIES, AND REPRODUCIBILITIES

Material Analyzed and Methods	Number of Analyses	Magnesium Mean ± Standard Deviation	Per Cent Recovery
		mEq./l.	
Pooled serum			
Colorimetric	10	1.50 ± 0.11	
Flame	10	1.56 ± 0.12	
Pooled serum with magnesium increased by 1.20 mEq. per l.			
Colorimetric	10	2.78 ± 0.12	106.7
Flame	10	2.80 ± 0.21	103.3
Pooled serum with magnesium increased by 2.4 mEq. per l.			
Colorimetric	10	4.00 ± 0.16	104.2
Flame	9	3.92 ± 0.04	98.3

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ESTIMATION OF MAGNESIUM IN SERUM BY MEANS OF FLAME SPECTROPHOTOMETRY

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Low intensity of emitted light of spectral oxide bands of magnesium has limited use of flame spectrophotometry as a method for accurate estimation of magnesium in biologic fluid. Occurrence of interference effects because of sodium, potassium, and phosphates results in serious error unless elaborate and time-consuming chemical pretreatment[1, 2] of the specimen is performed either to eliminate these ions or to add them to the sample in definitely known quantities.

A modification of the self-standardizing method of Rothe and Sapirstein[3] for calcium in biologic fluid has been adapted for the estimation of magnesium in serum. In the self-standardizing method, the luminosity value of a known quantity of magnesium under the conditions of interference present in the unknown serum is determined by measuring the increase in luminosity produced by addition of a standard amount of magnesium to the serum. The concentration of magnesium in the unknown serum is then determined from the ratio of luminosity of the unknown to luminosity of the known quantity of magnesium. Intensity of spectral emission is determined at a wave length of 372 mμ and background emission is determined at 360 mμ.

METHOD

Preparation of solutions. To each of 2 10-ml. volumetric flasks *A* and *B* is added 1 ml. of serum. To flask *B* is added 1 ml. of an aqueous solution containing 2 mEq. per l. of magnesium. The contents of each flask are diluted to the mark with distilled water or with 0.1 per cent sterox.

Instrumental data.

1. Instrument—Beckman model B spectrophotometer with flame attachment.
2. Photodetector tube—photomultiplier blue sensitive phototube.
3. Phototube setting—E (full sensitivity).
4. Sensitivity selector switch—4 position (full sensitivity).
5. Dark current control—adjusted to yield a reading of 0 per cent transmittance on the galvanometer scale when the 1:10 dilution of serum is aspirated at a wave length of 360 mμ.
6. Wave length—background luminescence is measured at 360 mμ; luminescence resulting from magnesium is measured at 372 mμ.
7. Slit width—0.3 mm.
8. Shutter control—up position (blue filter in position).
9. Oxygen pressure—10 to 15 psi. Adjustment is made to the point at which maximal variation in pressure produces minimal variation in per cent transmittance reading.
10. Hydrogen pressure—5 psi. Adjustment is made to the point at which maximal variation in pressure produces minimal variation in per cent transmittance reading.

Procedure.

1. While aspirating the 1:10 dilution of serum (*A*), with the shutter open (up position), and with the wave length setting at 360 mμ, the meter needle is adjusted to 0 per cent transmittance by varying the dark current control.
2. The wave length setting is then adjusted to 372 mμ. The per cent transmittance reading of serum (*A*) is determined under otherwise identical operating conditions as in Step 1.

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3. The per cent transmittance reading of the 1:10 dilution of serum plus magnesium standard (*B*) is then determined under identical operating conditions as in Step 2.

Calculation.

$$\frac{\text{Per cent transmittance of serum } A}{\text{Per cent transmittance of serum } B - A} \times \text{Magnesium standard in mEq. per l.} = \text{Serum concentration in mEq. per l.}$$

RESULTS

The mean value obtained in analysis of 25 normal serums was 2.04 mEq. per l. of magnesium with a standard deviation of 0.14 mEq. per l. Analysis of a replicate series of samples resulted in an average difference of 0.05 mEq. per l. with a standard deviation of 0.06 representing a maximal error within 2 standard deviations of ±5.9 per cent.

Recovery of added magnesium in aqueous solution ranged from 97.0 to 101.5 per cent with a mean of 98.8 per cent.

Effect of Interfering Substances

Addition of sodium (Table 1), potassium (Table 2), and glucose (Table 3) to serum results in potentiation of spectral emission of magnesium. Addition of calcium and urea produces no change while phosphates (Table 4) produce a depression of spectral emission. These interfering effects emphasize the difficulty in flame photometric analysis for magnesium involving comparison of luminosity of unknown serums to luminosity of a standard containing these ions in approximately the same concentration. These interfering effects are automatically eliminated by using the self-standardizing method in which the patient's own serum is used as medium for the standard. Addition of up to 4 mEq. per

TABLE 1

EFFECT OF ADDITION OF SODIUM TO SERUM ON APPARENT CONCENTRATION OF MAGNESIUM

Sodium Concentration in mEq. Per l.			Magnesium Concentration	Apparent Magnesium Concentration	Per Cent Error
Initial	Added	Total			
140		140.0	2.18		
140	1.4	141.4	2.18	2.18	
140	2.8	142.8	2.18	2.30	5.5
140	4.2	144.2	2.18	2.48	13.7
140	5.6	145.6	2.18	2.64	21.1
140	7.0	147.0	2.18	2.71	24.3
140	11.0	151.0	2.18	3.10	42.2
140	24.0	164.0	2.18	3.78	73.3
140	28.0	168.0	2.18	4.18	91.7

TABLE 2

EFFECT OF ADDITION OF POTASSIUM TO SERUM ON APPARENT CONCENTRATION OF MAGNESIUM

Potassium Concentration in mEq. Per l.			Magnesium Concentration	Apparent Magnesium Concentration	Per Cent Error
Initial	Added	Total			
4.0		4.0	2.1		
4.0	2.0	6.0	2.1	2.43	15.7
4.0	4.0	8.0	2.1	2.77	31.8
4.0	6.0	10.0	2.1	2.88	37.1
4.0	8.0	12.0	2.1	3.10	47.6
4.0	10.0	14.0	2.1	3.28	56.2
4.0	12.0	16.0	2.1	3.43	63.3

TABLE 3

EFFECT OF ADDITION OF GLUCOSE TO SERUM ON APPARENT CONCENTRATION OF MAGNESIUM

Glucose Concentration in mg. Per 100 ml.			Magnesium Concentration	Apparent Magnesium Concentration	Per Cent Error
Initial	Added	Total			
100	50	150	2.09	2.09	
100	100	200	2.09	2.10	0.5
100	150	250	2.09	2.16	3.4
100	200	300	2.09	2.23	6.7
100	300	400	2.09	2.38	13.9
100	400	500	2.09	2.40	14.8
100	500	600	2.09	2.84	35.9

TABLE 4

EFFECT OF ADDITION OF PHOSPHORUS TO SERUM ON APPARENT CONCENTRATION OF MAGNESIUM

Phosphorus Concentration in mg. Per 100 ml.			Magnesium Concentration	Apparent Magnesium Concentration	Per Cent Error
Initial	Added	Total			
4.0		4.0	2.04		
4.0	2.0	6.0	2.04	2.04	
4.0	4.0	8.0	2.04	2.04	
4.0	6.0	10.0	2.04	1.91	5.9
4.0	8.0	12.0	2.04	1.85	9.3
4.0	10.0	14.0	2.04	1.85	9.3

l. of potassium, 14 mEq. per l. of sodium, 5 mEq. per l. of calcium, 200 mg. per 100 ml. of glucose, 150 mg. per 100 ml. of urea, and 4 mg. per 100 ml. of phosphorus to both serum dilution (*A*) and serum dilution with added magnesium (*B*) resulted in no significant difference in final result obtained.

DISCUSSION

Intensity of spectral emission of magnesium at 372 mμ is approximately 0.1 per cent of that of sodium at 589.3 mμ. High phototube sensitivity, low sample dilution, and relatively wide slit widths are necessary for analysis. Adequate phototube sensitivity is achieved by means of a photomultiplier circuit. Low sample dilution increases viscosity of the sample compared to aqueous standards and increases the frequency of plugging of the aspirator assembly. The aspirator must be rinsed and mechanically cleaned frequently to prevent erratic readings. At this dilution, addition of increasing quantities of an aqueous standard magnesium solution produces a linear calibration curve (Fig. 1). A slit width of 0.3 mm. appears to yield optimal results but can be varied to conform with individual instrumental peculiarities. Decreasing the slit width decreases spread of the readings on the transmittance scale. Increasing the slit width intensifies background noise and increases difficulty in obtaining reproducible readings. At a slit width of 0.3 mm., 1 scale division on the per cent transmittance scale corresponds to approximately 0.22 mEq. per l. of magnesium.

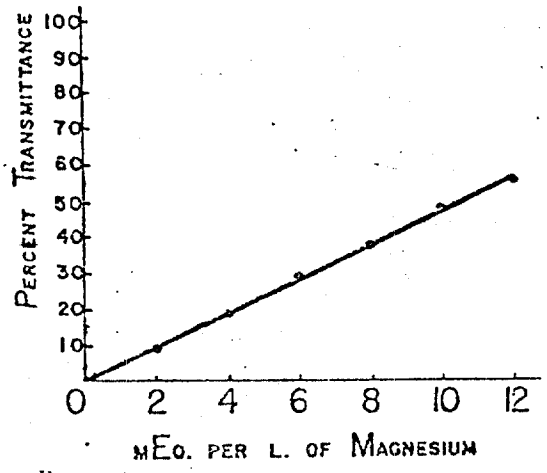

FIG. 1. The linear relation between transmittance and concentration of magnesium in serum is illustrated by means of the addition of standard amounts of magnesium to serum.

SUMMARY

1. A self-standardizing method for flame photometric estimation of magnesium is described.

2. Sodium, potassium, and glucose increase spectral emission, and phosphorus decreases spectral emission of magnesium. Urea and calcium had no effect on intensity of emitted light. These interfering effects are eliminated in the self-standardizing method.

3. Addition of up to 4 mEq. per l. of potassium, 14 mEq. per l. of sodium, 5 mEq. per l. of calcium, 200 mg. per 100 ml. of glucose, 150 mg. per 100 ml. of urea, and 4 mg. per 100 ml. of phosphorus produced no significant effect on the magnesium values obtained.

4. Mean value for serum magnesium in normal persons was 2.04 mEq. per l. with a standard deviation of 0.14.

5. Analysis of a replicate series of samples yielded an average difference of 0.05 mEq. per l. with a standard deviation of 0.06, representing a maximal error of ±5 per cent.

6. Recovery of added magnesium to serum varied from 97.0 to 101.5 per cent with a mean of 98.8 per cent.

SUMMARIO IN INTERLINGUA

1. Es describite un methodo auto-standardisante pro le estimation de magnesium per spectrophotometria a flamma.

2. Natrium, kalium, e glucosa augmenta le emission spectral de magnesium; phosphoro reduce lo. Urea e calcium habeva nulle effecto super le intensitate del lumine emittite. Iste interferentias es eliminate in le methodo auto-standardisante.

3. Le addition de usque a 4 mEq. de kalium per litro, 14 mEq. de natrium per litro, 5 mEq. de calcium per litro, 200 mg. de glucosa per 100 ml., 150 mg. de urea per 100 ml., e 4 mg. de phosphoro per 100 ml. produceva nulle significative effecto in le valores obtenite pro magnesium.

4. Le valor medie de magnesium in sero esseva pro subjectos normal 2.04 mEq. per litro, con un deviation standard de 0.14.

5. Le analyse de series replicate de specimens produceva un differentia medie de 0.05 mEq. per litro, con un deviation standard de 0.06. Isto representava un error maximal de ± 5 pro cento.

6. Le recovrage de magnesium addite al sero variava inter 97.0 e 101.5 pro cento. Le valor medie esseva 98.8 pro cento.

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A SIMPLIFIED FLAME SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF MAGNESIUM IN SERUM

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Several methods have been reported for the flame spectrophotometric estimation of magnesium in serum. Kapuscinski and co-workers[3] developed a method requiring wet ashing, evaporation, restoration of volume, and measurement of the 383 mμ oxide band luminosity. Davis[1] precipitated the magnesium as the 8-hydroxyquinolate, redissolved it in acetone-acetic acid mixture, and then measured the 285.2 mμ atomic line luminosity. Wacker and Vallee[7] adjusted the concentration of sodium of each serum to match that of the standard, diluted 1:5 without deproteinization, then measured the 371 mμ oxide band luminosity on a multichannel flame spectrometer. Teloh[6] presented a self-standardizing method that requires 2 samples, the addition of a known amount of magnesium to one of them, measurement of the luminosity at 360 mμ for zero background adjustment, and measurement of the 371 mμ luminosity for quantitation. Manna and co-workers[5] described a flame spectrophotometric method for magnesium in beverage alcohol. They found that the 80 per cent acetone solvent system enhanced luminosity so that the weaker but more selective atomic line, 285.2 mμ, could be used to advantage.

A flame spectrophotometric method for the determination of magnesium in serum is presented which involves fewer steps and is less cumbersome than those previously described. The method is based upon use of (1) trichloroacetic acid to release the protein-bound magnesium and to precipitate the proteins, (2) 80 per cent acetone as solvent system to increase sensitivity, and (3) atomic line, 285.2 mμ, luminosity measurement to achieve greater selectivity. The method compares favorably with the Clayton Yellow method for accuracy, reproducibility, and specificity, yet is considerably faster.

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METHODS

Flame spectrophotometric method. One milliliter of serum was deproteinized with 1 ml. of 10 per cent trichloroacetic acid and mixed. Eight milliliters of acetone were added, the tube sealed with parafilm, thoroughly mixed, and centrifuged. The clear supernatant fluid was decanted to a sample beaker just prior to aspiration into the flame. A micro modification consisted of proportionate reduction of volumes of reagents and sample using 0.1 ml. of serum. Other modifications should be suitable providing the final concentration of acetone is 80 per cent and that of trichloroacetic acid is 1 per cent. Evaporation was not a serious problem if the tubes were well sealed and the analysis completed within a reasonable time. Four or more of the stock standards were carried through the entire process, using 1 ml. of standard with each series of samples, regardless of whether samples were processed on the macro or micro scale.

The flame luminosity was measured on a Beckman DU spectrophotometer equipped with a model 9200 flame attachment and a model 4300 photomultiplier using an oxy-hydrogen flame. Gas pressures were adjusted to yield maximal luminosity. On our instrument it was found to be 5 p.s.i. for hydrogen and 7 p.s.i. for oxygen. The following instrument settings were used: sensitivity, 2 turns from the counter clockwise limit; check switch, 0.1; wavelength, 285.2 mμ; slit-width, 0.06 to 0.08 mm.; photomultiplier sensitivity, 4; and zero suppression, off. The dark current was adjusted to zero with the

lated from these values are listed in Table 2. The 10 replicate analyses were performed both colorimetrically and flame spectrophotometrically for comparison of methods. The 2 methods are in satisfactory agreement. The average difference between duplicates, irrespective of the range of concentration, was 0.14 mEq. per l. for the colorimetric method and 0.06 mEq. per l. for the flame method.

The mean value for 73 samples of serum that were collected at random from the clinical laboratory was found to be 1.67 mEq. per l. with a standard deviation of 0.17. The normal range estimated as ±2.58 standard deviations was 1.23 to 2.11 mEq. per l.

Several other factors concerning the method were studied. The concentration of acetone in the final solution could be varied between 78 and 82 per cent without effect on the values obtained. The per cent of trichloroacetic acid could be increased or decreased by a factor of 2 without effect on the values obtained. The standard solutions diluted in acetone were found to be stable for only a few days, after which time some of the constituents began to precipitate. Dilutions of 1:5, 1:10, and 1:20 yielded the same results, the only difference being in the slope of the standard curve.

DISCUSSION

Inasmuch as the luminosity of magnesium is weak, maximal sensitivity was sought by combining the use of the photomultiplier tube with enhancement of emission by acetone while holding dilution to a minimum. In addition to increasing sensitivity, the 80 per cent acetone solvent system also increased flame stability. The atomic line, 285.2 mμ, was selected in preference to either oxide band, despite weaker emission, because it was found to be less susceptible to radiation interferences.

Efforts to shorten the method by omitting the trichloroacetic acid deproteinization step were unsuccessful, inasmuch as low and erratic values resulted. This apparently resulted from the magnesium being carried down with the protein precipitate when the acid was omitted.

The time required to analyze a series of samples by the flame method was found to be approximately one-half that required by the colorimetric method. Otherwise, the 2 methods were in agreement, both showing satisfactory recovery and approximately equal reproducibility.

SUMMARIO IN INTERLINGUA

Es presentate un methodo de spectrophotometria a flamma pro le determination de magnesium in sero. Illo require minus stadios e es minus complexe que le methodos previemente describite. Illo es basate super (1) le uso de acido trichloroacetic pro liberar le magnesium ab su ligation a proteina e pro precipitar le proteina, (2) le uso de 80 pro cento de acetona como systema solvente pro augmentar le sensibilitate, e (3) le uso de mesurationes de luminositate al linea atomic de 285.2 mμ pro obtener un plus grande selectivitate. Le methodo se compara avantagiosemente con le methodo colorimetric a jalne titan con respecto a accuratia, reproducibilitate, e specificitate, sed illo es considerabilemente plus rapide.

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Potentiometric Determinations of Calcium, Magnesium, and Complexing Agents in Water and Biological Fluids

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Calcium is determined accurately, even in multicomponent solutions, by direct titration with a solution of the tetrasodium salt of EGTA at a pH of about 8.5. A recording pH meter indicates a sharp upward break at the equivalence point, unaffected by a large excess of magnesium in the presence of citrate. Continued titration with tetrasodium EDTA of the same sample shows a break in pH at the equivalence point for magnesium. The limit of detection for both metals is 0.5 μg in samples up to 100 ml. More than 10 μg of EDTA or EGTA can be determined by addition of excess cadmium and back-titration with tetrasodium EGTA at pH 4.5 to 5.0. Reproducibility and accuracy are better than ±0.5% for the samples containing more than 0.5 μmole of the metals or ligands.

Difficulties in end point detection of chelometric titrations in turbid or colored biological fluids can be avoided by potentiometric detection of the equivalence point. Schmid and Reilley (*1*) used a mercury electrode and some mercuric chelate in their samples of pure salt solutions for determination of calcium. This method, however, is limited by interferences in practical solutions—*e.g.*, redox potential and chloride ion.

Titrations of calcium plus magnesium can be done with the tetrasodium salt of EDTA under conditions that a change in pH indicates the end point. Schwarzenbach and Biedermann (*2, 3*) did such titrations of pure salt solutions. Their results were not promising, and they discontinued this approach. A search for colored indicators was expanded and continues to the present.

We shall present data showing why the pH change approach did not give better results. Conditions will be given under which sharp end points are obtained, even in urine and blood plasma, using readily available equipment.

EXPERIMENTAL

Apparatus. Leeds and Northrup microelectrodes, or any type of combination pH electrodes, are used in 50- or 100-ml beakers containing magnetic stirrers. The pH measurements are made with a multispeed recording pH meter (Heath EUW 301-M). Either one of two Sargent constant rate burets is attached to the outlet which is only energized when the recorder operates.

Reagents. Deionized distilled water is used in the preparation of the solutions which are kept in polyethylene containers.

Ethyleneglycolbis-(betaaminoethylether)-*N,N'*-tetraacetic acid (or EGTA, Eastman Cat. No. 8276) is dissolved by addition of an accurately measured volume of sodium hydroxide solution to make pH about 7.0. Its tetrasodium salt is prepared by addition of another identical volume of hydroxide, and a stock solution of 0.1*M* is made.

Disodium ethylenediaminetetraacetate (Fisher Scientific Co.) is converted to tetrasodium EDTA with a calculated amount of 50% sodium hydroxide, and diluted to 0.1*M*. The proper stoichiometry is checked by titration of a standard calcium solution (see procedures).

Trisodium citrate and cadmium nitrate, each about $5 \times 10^{-3}M$ solutions, are used.

Procedures. Calcium. Samples preferably containing more than 20 μg of calcium are diluted to about 20 ml and adjusted to pH 8.0 to 8.6 with dilute strong acid or base. Urine and blood plasma can be titrated without further preparation. For poorly buffered samples—*e.g.*, distilled water—and in the presence of an excess of magnesium, or when a precipitate is formed on adjustment of pH, the addition of 2 or more ml of $5 \times 10^{-3}M$ trisodium citrate is recommended. Samples containing a large excess of magnesium should be titrated in a pH region of about 8.2 or below.

The titrant is 5×10^{-4} to $10^{-3}M$ tetrasodium-EGTA added at a rate of 0.96 ml/min. During the titration of calcium, little change of pH is observed. The titration is discontinued after a sharp break occurs in the pH plot, and sufficient excess of titrant (about 0.5 ml) is added to allow precise interpolation.

The sum of dead time and calcium content of water and citrate solution is obtained by performing a blank titration using the same amount of water and citrate as in the sample. The dead time is found by restarting the buret and recorder about 1 min after completion of the titration. The distance on the plot before pH rises again represents the delay between start and response of the electrode. Blank and dead time can be determined with high precision using a high speed of the recorder.

Standardization of tetrasodium-EGTA or -EDTA solutions is done by titration of a standard calcium solution ob-

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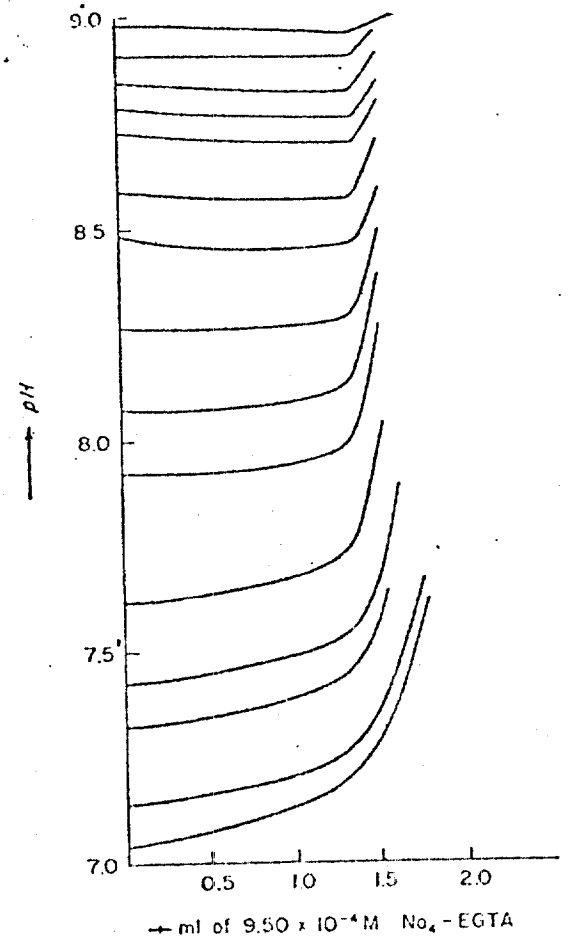

Figure 1. Titration plots of calcium with tetrasodium–EGTA, in the presence of citrate, as a function of the initial pH of sample

ained by dissolving calcium carbonate in nitric acid, and dition of 2 ml of $5 \times 10^{-3}M$ citrate solution.

MAGNESIUM. After completion of the calcium titration, hange to the buret containing 5×10^{-4} to $10^{-3}M$ tetrasodium–EDTA, and titrate without adjustment of pH of sample, because a break in the pH plot occurs in the same pH region as in the calcium titration. After correction for dead time, the EDTA titration represents magnesium.

A less accurate but more convenient measure of magnesium in some samples can be obtained by adjustment of pH to 9.2 to 9.3 after the equivalence point for calcium is reached without addition of citrate. Continue titration with tetrasodium–EGTA. A small bend upward in pH indicates the equivalence point for magnesium. The amount of EGTA used between the final equivalence point and the one for calcium represents magnesium in the sample. Such a titration is not possible in blood plasma or samples containing citrate and other complexing agents. Also, an additional blank may be introduced by the base used for pH adjustment.

EGTA or EDTA. A measured volume of excess of cadmium nitrate solution is added to the sample that should not contain heavy metals. After adjustment of pH to 4.5 to 5.0, a titration is done with tetrasodium-EGTA to a clear break in the pH plot. The titration value in absence of complexing agent is found by titration of a same volume of cadmium solution which should contain a small amount of buffering agent, such as citrate or acetate. The difference in back-titrations is equivalent to the amount of ligand in the sample.

Total calcium is obtained by adjustment of pH to 8.0 to 8.6 after the end point for the cadmium titration is reached. Continue titration with tetrasodium-EGTA. Calcium is

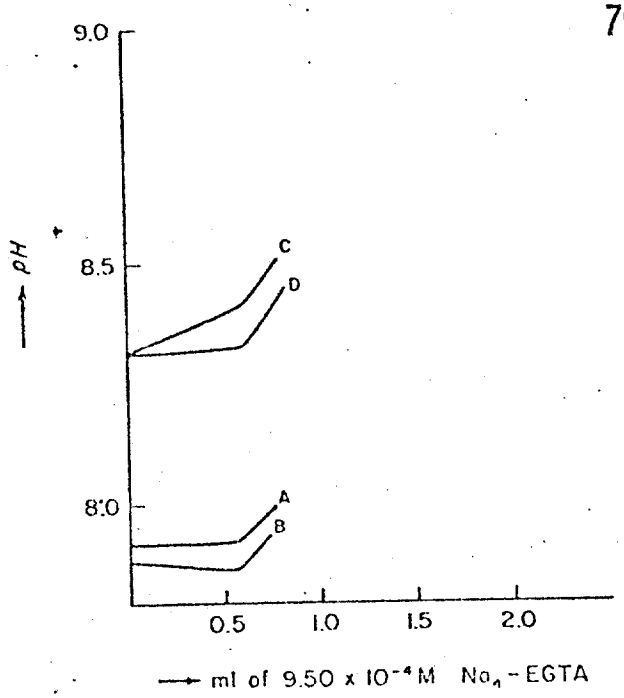

Figure 2. Titration of serum samples

A. 0.2 ml of rat serum
B. Rat serum with citrate
C. 0.2 ml of human serum
D. Human serum with citrate

calculated from the distance between equivalence points at pH 4.5 to 5.0 and at 8. Contamination of reagents should be checked by appropriate titrations of blanks.

RESULTS

Optimum pH Range of Samples. Figure 1 represents a series of titration curves obtained from samples prepared by diluting 0.200 ml of $6.16 \times 10^{-3}M$ calcium chloride and 2 ml of $5 \times 10^{-3}M$ trisodium citrate to 20 ml and adjusting an initial pH between 7 and 9. The titrations were carried out with $9.5 \times 10^{-4}M$ tetrasodium–EGTA in the Sargent buret.

Direct reading of the equivalent point without interpolation is possible at pH 8.6 and above. The top curve represents a sample that also contained 1.00 ml of $2.1 \times 10^{-3}M$ magnesium nitrate. At pH values of about 8.0, interference of magnesium becomes virtually nil in the presence of citrate. Without citrate, an upper limit of about pH = 8.6 is necessary in the presence of moderate amounts of magnesium, and sharp equivalence points for calcium are obtained down to pH = 7.5.

Biological samples give sharper end points at lower pH regions than calcium citrate solutions, down to pH = 8.0. Figure 2 shows some examples of urine and serum titrations, as well as the effect of citrate addition to serum on the plot.

Magnesium determinations can be performed, but excess of citrate must be avoided, and an initial pH of 9.2 to 9.3 is required to determine magnesium in urine with tetrasodium–EGTA. A superior end point is obtained in the titration of calcium plus magnesium with tetrasodium–EDTA at pH = 8.2 (Figure 3).

Improper adjustment of the stoichiometry of the tetrasodium ligand shows up either in excessive increase or decrease of pH during the titration. However, a sharp end point is obtained anyway, provided this point remains in the optimum pH region, as shown in Figure 3, in which the tetrasodium–EDTA contained some trisodium salt. Determinations of

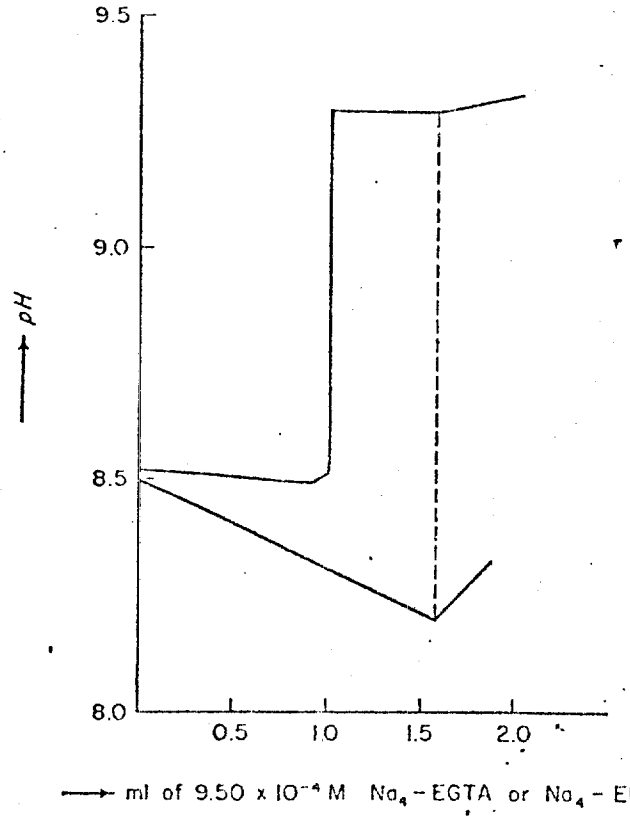

Figure 3. Determination of calcium and magnesium in 0.100 ml of human urine

Top curve, titration with tetrasodium–EGTA, pH adjusted to 9.3 after end point for calcium. Bottom curve, titration with tetrasodium–EDTA in the presence of citrate

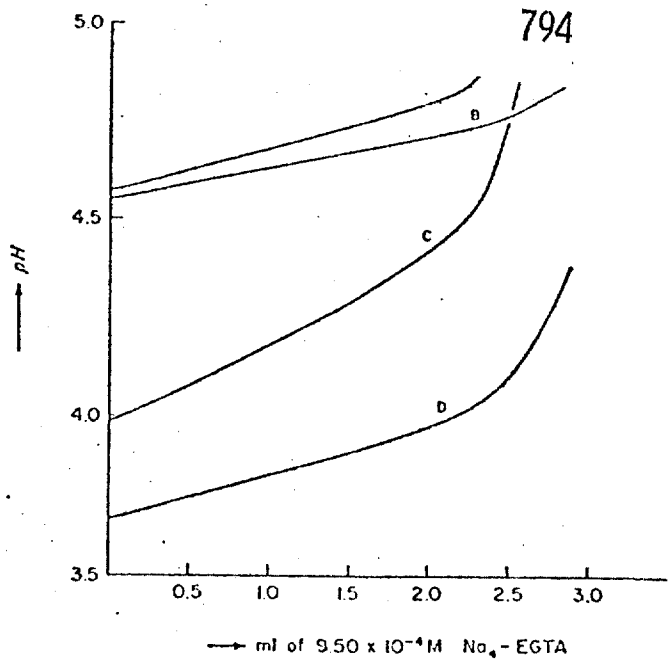

Figure 4. Determination of EGTA in rat urine

A. Back-titration after addition of 0.400 ml of cadmium solution to 0.100 ml of urine
B. Direct titration of 0.400 ml of cadmium solution plus 2 ml of $5 \times 10^{-3} M$ citrate plus 1.0 ml of $6 \times 10^{-3} M$ calcium chloride
C and *D.* Same as *B*, but without citrate

EGTA and EDTA in rat urine show clear equivalence points for excess of cadmium at pH between 4.5 and 5.0. Titration curves of cadmium and of rat urine after addition of excess of cadmium are given in Figure 4. Interpolation of the plots is facilitated by the presence of buffer, such as acetate or citrate. Direct titration of acid cadmium solutions without buffer gave ambiguous poor equivalence points (curves *C* and *D*).

Precision, Accuracy, and Limit of Detection. In titration of a variety of prepared solutions varying in volume from 20 to 25 ml, the titrant was added at a rate of 0.96 ml/min. The speed of the recorder was varied from 5 sec/inch for samples of low calcium content to 15 or 30 sec/inch for standard solutions. Table I gives the results expressed in micrograms of calcium, magnesium, or cadmium as obtained by the various methods, the range of results of quadruple determinations, and the deviation of the average results from the amount taken. Calculations were made from distances on the chart after correction for blanks.

Table I. Titrations of Standard Solutions

Taken, μg	Foreign ion, μg	Citrate, ml	Method[a]	End point, pH	Found, μg	Range, μg	Error, %
0 Ca	...	2	A	7.5–8.8	1.30	0.1	...
0 Ca	...	10	A	8.0–9.0	1.40	0.1	...
160 Ca	...	0	A	7.8–8.8	158.5	0.4	−1.0
160 Ca	...	2	A	8.0–8.8	160	0.3	Ref.
160 Ca	...	10	A	8.2–8.8	160	0.3	0
160 Ca	79.5 Mg	2	B	8.0–8.4	160	0.4	0
79.5 Mg	160 Ca	2	B	8.7–8.8	79.9	0.4	+0.5
0 Ca	82.5 Mg	2	B	8.3–8.5	0.2	0.2	...
82.5 Mg	0 Ca	2	B	8.7–8.9	82.8	0.2	+0.25
40 Ca	134 Mg	0	A	7.8–8.0	39.8	0.2	−0.5
134 Mg	40 Ca	0	A	9.3–9.5	133	1.0	−0.75
134 Mg	40 Ca	2	B	8.8	134	0.4	0
134 Mg	40 Ca	2	C	8.6–8.8	134.5	0.3	+0.4
80 Ca	200 Cu	5	A	8.8	80		0
80 Ca	300 Ba	2	A	7.5	80.5		+0.25
80 Ca	300 Sr	2	A	7.4	80.2		+0.3
105 Cd	800 Ca	2	D	4.8	105		0
210 Cd	400 Ca	2	D	4.9	210		0
80 Ca	210 Cd	2	D	4.8; 8.2	80		0

[a] A, Ca by titration with Na_4–EGTA; Mg by titration with same reagent (pH 9.2–9.4). B, Ca same as in A, followed by Mg titration with Na_4–EDTA. C, Ca plus Mg with Na_4–EDTA in one aliquot; Ca as in Method A in other aliquot. D, Cd with Na_4–EGTA at pH 4.5–5.0; Ca by continued titration at pH 8.0–8.8.

Table II. Comparison of Results in Practical Solutions

Sample	Titration method[a]	Sought	Found, in ppm, by Titrations	Found, in ppm, by Atomic absorption[b]
Human urine	B	Ca	143	143.3
	B	Mg	92	90.4
man plasma	B	Ca	79	78.5
	B	Mg	20	19
y water, Richmond, Va.	B	Ca	18.2	18.2
	B	Mg	2.89	2.91
	A	Mg	2.78	2.91
Atlantic Ocean, Eastern Shore	B	Ca	381	381.5
	B	Mg	1180	1131
	A	Mg	1170	1131
	C	Mg	1178	1131
Human plasma, +162 μg of EDTA	D	EDTA	164	...
		Ca	79	78.5
Human plasma, +190 μg of EGTA	D	EGTA	190	...
		Ca	79	78.5
Human urine, +162 μg of EDTA	D	EDTA	162	...
		Ca	143	143.3
Human urine, +190 μg of EGTA	D	EGTA	192	...
		Ca	143	143.3
Magnesium nitrate crystals	A	Ca	123	106

[a] Same as in Table I.
[b] Perkin Elmer, Model 303, in presence of lanthanum, in addition EDTA for sea water.

The presence of citrate caused a slight increase in the recorded use of EGTA and EDTA. Recrystallization of the EGTA did not change this increase significantly. Standardizations of EGTA and EDTA were always done in the presence of citrate.

Table II presents results of practical solutions by the titration methods and by atomic absorption. In addition, the quantitative recovery of complexing agents from blood plasma and urine samples was verified.

The limit of detection under our experimental conditions is below 0.5 μg calcium or magnesium. This amount of calcium could be detected in 100 ml of deionized water after leaving it in a borosilicate flask for one week. The detection limit for EDTA and EGTA is about 10 μg, and a range of 4 μg is obtained.

Interferences. As both EDTA and EGTA are strong complexing agents for multivalent metals, some of these metals are titrated with calcium, even in the presence of masking agents such as citrate. Citrate eliminates interference of copper and iron, but not of zinc. A small excess of cyanide masks the last metal, but the calcium titration should be carried out above pH = 8.6 with citrate added to prevent interference of magnesium.

Table I shows some examples of solutions containing strontium and barium which usually interfere in titrations of calcium. If titrations are carried out to an equivalence point at pH 7.5 or below, calcium is determined without difficulty in the presence of up to 350 μg of total barium and strontium in the presence of citrate.

Solutions of high buffer capacity in the optimum pH range for titrations give a poorly defined end point. A maximum of 0.5 mmole of phosphate is allowed for precise end point detection for calcium at pH 8.6 or above. Therefore, a phosphate/calcium mole ratio of 1000 is allowed in the determination of 20 μg of calcium.

Although magnesium can be determined by titrations of aliquot samples, one with EDTA and one with EGTA, the difference between equivalents used may be small compared to the total titrations. It is more convenient to titrate magnesium with EDTA in a sample in which calcium has been titrated with EGTA to slightly beyond the equivalence point. A large excess of EGTA obscures the end point of the EDTA titration, because it reacts partly with magnesium, causing a rise in pH before the equivalence point. The last entry in Table II shows that traces of calcium can be determined in a large excess of magnesium. The titration is carried out in solutions containing sufficient citrate to complex magnesium. Samples of 0.50 and 1.00 g of magnesium nitrate crystals were used in the titrations.

DISCUSSION

The suggested titration methods for calcium and magnesium give titration plots from which equivalence points can be determined with high precision. The reason for the favorable plots is the essentially complete complexation at the equivalence point in the relatively high pH region, shown in Figure 1 for calcium, in contrast with pH regions shown previously (2).

Major advantages of the method are apparent in samples containing a large excess of magnesium over calcium. Even trace amounts of calcium can be determined in magnesium nitrate, although the high titration value, compared to atomic absorption, may indicate the presence of heavy metals which are determined with calcium.

The titration method gives closer to accepted values for magnesium in sea water than atomic absorption does, and good agreement is found for calcium by the two methods.

Sometimes it is necessary to perform titrations at lower pH than at optimum range. In such cases precise interpolation is facilitated by the presence of a small amount of buffer. Therefore, biological fluids can be titrated over a wider pH region than pure salt solutions. In addition, interference of up to 350 μg of total strontium and barium can be avoided by titration at pH 7.5 or below, and the sum of these metals can be found later by titration at a higher pH.

Simplification of apparatus is possible in titrations of relatively larger amounts of calcium. Manual titrations can be carried out using a regular pH meter as the indicator. Sharp end points are obtained if $10^{-2}M$ or stronger titrants are used. A color indicator is less suitable because strict requirements are imposed on the stoichiometry of the titrant because the pH of the sample is not allowed to change significantly during the titration.

The authors thank Charles O'Rear f[or] 794 [abs]orp-tion measurements, and Henry P. Lau f[or] tudies.

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NOTES

Gas Chromatographic Analysis of Pyruvic and α-Ketoglutaric Acids

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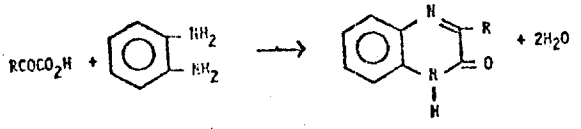
CONVERSION of pyruvic and α-ketoglutaric acids to their methyl esters by acid-catalyzed methanolysis or reaction with diazomethane can lead to two products. Simmonds, Pettitt, and Zlatkis (*1*) in a thorough study have shown that, in addition to the desired ester, the ketal ester is formed in methanolysis and a glycidate ester is formed with diazomethane. These authors have reviewed previous investigations of the gas chromatography of pyruvic and α-ketoglutaric acids (*1*). In addition to competing reactions during its formation, at least in the case of pyruvic acid, the methyl ester presents a handling problem. Because of its relatively high volatility, a sample containing the ester can be fractionated prior to gas chromatography.

We report an approach to the gas chromatography of these keto acids that relies on the formation of quinoxalones (Equation 1).



(1)

Infrared and nuclear magnetic resonance spectral studies of these heterocyclics showed they existed, to the limits of detection, entirely in the keto form and not in the hydroxyquinoxaline enol form.

This derivative has the advantages of rapid formation, low volatility, and a large number of reduced carbons and, therefore, greater detectability using flame ionization. Furthermore, the quinoxalone's mild acidity permits pH control for separation from basic, neutral, or strongly acidic constituents of biological samples.

EXPERIMENTAL

Apparatus. A Barber-Colman Model 5000 gas chromatograph equipped with a hydrogen flame ionization detector was used. The chromatographic columns were 6-foot U-tubes packed with 80/100 acid-washed Chromosorb W coated either with 13% SE-30 or 1% OV-17.

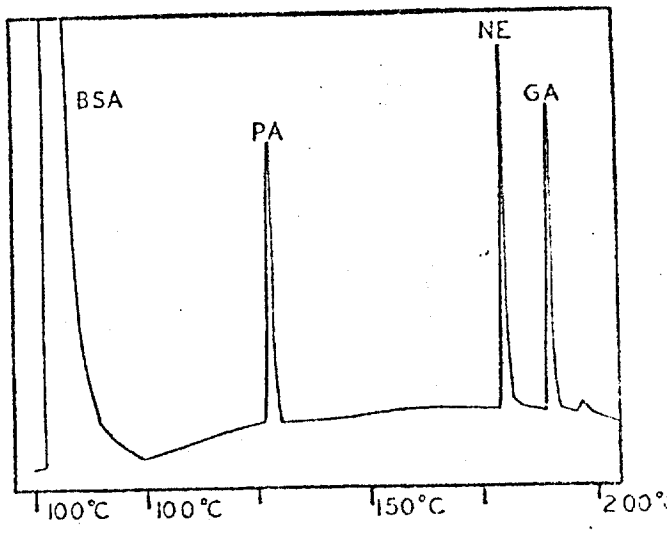


Figure 1. Chromatogram of the trimethylsilylated quinoxalo[ne] derivatives of pyruvic acid, PA, and α-ketoglutaric acid, G[A] and internal standard *p*-nitrophenyl phenyl ether, NE

Reagents. Quinoxalones of pyruvic and α-ketoglut[aric] acids were prepared by mixing a solution of 60 mmol sublimed *o*-phenylenediamine in 100 ml of 10% acetic a[cid] with a solution of 30 mmole of keto acid in 30 ml of water (The precipitated quinoxalone was filtered after 15 m[in] washed with water, dried, and recrystallized from methan

Under the dilute solution conditions found in biologi[cal] samples, pyruvic and α-ketoglutaric acids can be conver[ted] to quinoxalones in 100% yields.

Standard solutions prepared to concentrations known better than 1% contained about 0.1 g solute/10 ml dry py[ri]dine. Solutes were 3-methyl-2-quinoxalone (from pyru[vic] acid), 3-(2-carboxyethyl)-2-quinoxalone (from α-ketoglut[aric] acid), 6-methyl-2-naphthol, and *p*-nitrophenyl phenyl eth[er]

Procedure. The above-mentioned pyridine solution quinoxalone was mixed in varying amounts, 5–50 μl, with μl of the pyridine solution of either the naphthol or ni[tro] ether mentioned above. To this was added 200 μl of (trimethylsilyl) acetamide (BSA) and 50 μl of pyrid[ine]

(1) P. G. Simmonds, B. C. Pettitt, and A. Zlatkis, ANAL. CHEM., 38, 163 (1967).

(2) D. C. Morrison, *J. Amer. Chem. Soc.*, 76, 4483 (1954).

weeks. Nor were viable organisms recovered after they had been exposed to the remaining two paint-sterilant samples which had been stored for 1–4 weeks. Curiously, a few viable spores were recovered from specimens exposed to the latter samples when the fresh mixtures were used. In these cases, however, lethal action of the paint mixtures was still very good with a kill of 10^5–10^6 spores per specimen.

To be certain of the validity of the interpretation of the sporocidal properties of the paint-sterilant mixture, two control tests were necessary: a spore recovery test and a residual formaldehyde inhibition test. In the spore recovery test, coupons were inoculated and painted as in the sterilization test except that the paint formulation contained no formaldehyde. The dried coupons were placed in sterile water and the paint removed by ultrasonic scrubbing. Dilutions, pour plates and plate counts were made to determine the spore recovery. The results (Table 1, spore recovery control) showed that all spores deposited on the test specimens were recoverable. Thus, any viable micro-organisms remaining after exposure to the paint-sterilant mixture would be recoverable.

The residual formaldehyde inhibition test was conducted to determine whether residual formaldehyde transferred to the growth medium with the test dilution aliquots would have a bacteriostatic effect on the growth of viable micro-organisms. Aluminium coupons were prepared as in the sterilization test except that no spores were introduced on to the coupons. The coupons were painted with 0·1 ml. of the formaldehyde-thermal coating. Each dried coupon was aseptically placed into 10 ml. sterile distilled water and the paint removed ultrasonically. 1,000 spores of *B. subtilis* were added to each prepared dilution. 1-ml. aliquots were removed to prepare pour plates with tryptone glucose extract agar. The plates were incubated for 48 h at 37° C, after which plate counts were made.

The results (Table 2) show no inhibition of bacterial growth by formaldehyde residue in the growth medium. The entire population of micro-organisms added (10^3) produced colonies within 48 h.

[1] Phillips, Charles R., and Hoffman, Robert K., *Science*, 132, 991 (1960).
[2] *Science*, 128, 887 (1958).
[3] Varga, R. J., *Surveyor Spacecraft System Final Sterilization Report*, 1, *SSD 3372R* (June 1963).

ACCURACY OF DETERMINATIONS OF SERUM MAGNESIUM BY FLAME EMISSION AND ATOMIC ABSORPTION SPECTROMETRY

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RECENT studies in this laboratory have demonstrated the pertinence of the Beer–Lambert Law to atomic absorption spectroscopy†. As a result, a marked increase in analytical sensitivity has been obtained by directing the flame through a horizontal, tubular absorption cell, thereby elongating the length of the absorption path. The development of exceedingly sensitive atomic absorption spectrometers for routine analytical purposes is a direct product of this work.

There has been some uncertainty concerning the true concentration of magnesium in human serum resulting in some ambiguities concerning the significance of measurements of this element in pathological states[1–3]. It seemed important therefore to examine this problem critically and provide data with suitable and sensitive methods.

The present communication describes a method for the measurement of magnesium in human serum utilizing a long flame path design. In these circumstances magnesium can be measured directly in 1 : 50 aqueous dilution of serum. Accuracy is has been determined by measurements of the U.S. National Bureau of Standards samples. In addition, a direct comparison of measurements made by atomic absorption spectroscopy with those obtained using a multichannel flame spectrometer has been carried out on both certified National Bureau of Standards materials and serum. These investigations provide, for the first time, verified data on accuracy of two methods employed for the analysis of magnesium in biological fluids. They demonstrate that average normal serum contains 2·1 mequiv. of magnesium per litre[4], as was previously demonstrated by flame spectrometry[5].

The light source was a hollow cathode discharge tube, type 22604 (Westinghouse Electric Corp.). The light from the hollow cathode lamp was collimated by a quartz lens of length 15 cm placed 5·5 cm from the tube window; it passed through the absorption cell and was focussed on the entrance slit of the monochromator. The distance between the lens and the entrance slit was 71·5 cm. Morganite recrystallized alumina tubing, having an inside diameter of 1·9 cm and a length of 25 cm. was used as the absorption cell. It was mounted as previously described[4].

The sample was sprayed into the absorption cell by means of a Beckman atomizer burner (No. 4020). The fuel was a mixture of commercial hydrogen at a pressure of 2·8 lb./in.^{2} and air at a pressure of 10 lb./in.^{2}. The flow-rate of the sample through the burner was 1·5 ml./min.

The entrance and exit slits of the Zeiss monochromator, *M4QIII* were 0·03 mm wide; the latter was set at 2852 Å. A multiplier photo-tube (R.C.A. *1P28*) detected the absorption of radiation. Circuits for the high-voltage power supply and amplifier were virtually identical to those used in a multichannel flame emission spectrometer[4]. The output signal of the amplifier was displayed on a direct current microammeter[4].

A multichannel flame spectrometer with automatic background correction was used for measuring magnesium by flame emission[5]. The sample was aspirated and excited with a Beckman burner and an oxy-hydrogen flame. The magnesium line at 2852 Å was employed for all measurements.

Standard solutions were prepared from rods of magnesium metal (Johnson Matthey, Inc., London) dissolved in metal-free 6 N hydrochloric acid and diluted in water which had been purified by passage over a mixed bed ion exchange resin and then distilled in all-glass stills to yield a standard solution containing 10,000 p.p.m. of magnesium. Concentrated stock solutions were diluted further with metal-free distilled water. The concentrations utilized for working standards varied from 0·1 to 1 p.p.m.; these must be prepared just prior to use.

For atomic absorption measurements, serum samples were diluted fifty times with metal-free distilled water. Serum samples measured by flame emission were diluted four-fold with redistilled 5 per cent trichloroacetic acid (TCA).

National Bureau of Standards base alloy labelled National Bureau of Standards No. 171 and dolomite

* Investigator, Howard Hughes Medical Institute.

† The terminology of accuracy, repeatability, etc., is that suggested by the American Chemical Society as recorded by Hughes, H. N., *et al.* (*Anal. Chem.*, **24**, 1498 (1952)).

Table 1. Repeatability* of Magnesium Standards Used for Atomic Absorption Working Curve

Mg concentration (p.p.m.)	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Mean ± S.D. (p.p.m.)	C.V. (per cent)
0·1	0·046	0·052	0·052	0·052	0·052	0·052	0·051 ± 0·0027	5
0·3	0·174	0·171	0·163	0·163	0·163	0·163	0·165 ± 0·0041	2
0·5	0·326	0·301	0·290	0·312	0·296	0·296	0·305 ± 0·0109	3
0·7	0·412	0·412	0·376	0·438	0·426	0·414	0·413 ± 0·0163	4
1·0	0·584	0·602	0·554	0·584	0·602	0·584	0·585 ± 0·0111	2

* Each concentration of the standard solution was measured successively by the same analyst.

National Bureau of Standards No. 88 served as the certified standards of accuracy. These were dissolved in metal-free 6 N hydrochloric acid and diluted with metal-free distilled water.

A typical working curve for standard solutions of magnesium of 0·1, 0·3, 0·5, 0·7 and 1·0 p.p.m. obtained by atomic absorption measurements is shown in Fig. 1*A* and measurements of repeatability obtained on standards are recorded in Table 1. The coefficient of variation (*C.V.*) of 6 replicate readings varies from 4 per cent at 0·1 p.p.m. to 2 per cent at 1 p.p.m.

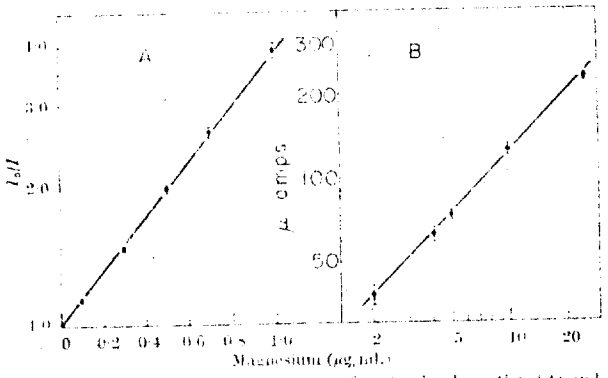

Fig. 1. Magnesium calibration curves for atomic absorption (*A*) and flame emission (*B*) analysis. The bars indicate the standard deviations of measurements at the indicated concentration

The accuracy of the method was determined by measuring National Bureau of Standards base alloy standard No. 171. One analyst performed seventeen measurements of the same standard known to contain 0·954 p.p.m. of magnesium over a period of two weeks (Table 2). The value obtained was 0·956 ± 0·035 p.p.m. with a coefficient of variation of 3·7 per cent. The overall accuracy of the mean of these 17 determinations was 100·3 per cent. National Bureau of Standards No. 88 was also analysed. The certified known value was 0·647 p.p.m. The mean of 13 measurements was 0·664 ± 0·18 p.p.m., equivalent to 102·6 per cent of the expected value.

The repeatability of measurements of magnesium as well as its recovery were determined on a 1 : 50 dilution of two normal sera to which 0·2 μg. of the element were added (Table 3). The coefficient of variation for 6 replicate

Table 2. Accuracy of Magnesium Analyses by Atomic Absorption: National Bureau of Standards No. 171 Base Alloy

Sample	Magnesium content (p.p.m.)
1	0·92
2	0·98
3	0·92
4	0·95
5	0·95
6	1·05
7	0·905
8	0·92
9	0·96
10	0·92
11	0·92
12	0·96
13	0·96
14	0·98
15	0·95
16	1·06
17	0·95
Mean	0·956 ± 0·035 *C.V.* 3·7 per cent
Certified Known Value of National Bureau of Standards No. 171	0·954 Accuracy 100·3 per cent

determinations on these sera was 1·7 and 4 per cent respectively. In each instance the recovery of added magnesium was 99 per cent.

Table 3. Repeatability and Recovery of Magnesium in Normal Serum measured by Atomic Absorption

Sample	No. of analyses	Magnesium Mean ± S.D. (m.equiv./l.)	Coefficient of variation (per cent)	Recovery (per cent)
Serum *A*	6	2·30 ± 0·04	2·2	
Serum *A* + 0·2 m.equiv. Mg	6	3·00 ± 0·08	1·7	99
Serum *B*	6	2·38 ± 0·08	4·0	
Serum *B* + 0·2 m.equiv. Mg	6	3·17 ± 0·12	3·8	99

Triplicate analyses of serum obtained from 10 normal individuals were also carried out (Table 4). A mean value of 2·14 ± 0·17 m.equiv./l. was obtained.

Table 4. Precision of Serum Magnesium Concentration of Ten Normal Persons as Measured by Atomic Absorption Spectroscopy

Sample No.	Magnesium (m.equiv./l.) 1	2	3
1	2·10	2·05	2·14
2	2·02	2·11	2·39
3	2·26	2·39	2·33
4	2·02	2·22	2·30
5	1·84	1·85	1·89
6	2·10	2·14	2·22
7	2·18	2·22	2·39
8	2·02	1·98	2·06
9	1·89	1·98	2·06
10	2·30	2·30	2·39

Mean ± *S.D.*: 2·14 ± 0·17. *C.V.* 8 per cent.

A typical calibration curve using working standards for flame emission is also shown in Fig. 1*B*. The repeatability varied from 7 per cent at 2 p.p.m. to 2 per cent at 25 p.p.m. The accuracy of the emission method as judged by measurement of National Bureau of Standards standard No. 171 was 98 per cent. Eleven determinations on the standard known to contain 9·54 p.p.m. gave a value of 9·36 ± 0·32 p.p.m. The certified value of National Bureau of Standards No. 88 (dolomite) is 6·47 p.p.m. ± 0·30 p.p.m. The mean of 11 measurements was 6·64 or 102·6 per cent of the known value.

The repeatability of measurements of the serum magnesium by flame emission spectroscopy of 2 normal persons was 2·21 ± 0·08 m.equiv./l. and 2·17 ± 0·12 m.equiv./l. The recovery of the magnesium added to each of these sera was 98 and 99 per cent respectively.

Table 5 compares directly simultaneous measurements of the magnesium concentration of seven serum samples both by atomic absorption and by flame emission spectrometry.

Table 5. Comparison of Normal Serum Magnesium Concentration measured by Atomic Absorption and by Flame Emission Spectroscopy

Sample	Magnesium concentration (m.equiv./l.) Emission	Absorption
1	1·96	2·10
3	2·34	2·35
5	1·87	1·85
6	2·04	2·15
7	2·19	2·26
9	1·93	1·98
10	2·14	2·33

The pioneering efforts of Walsh[6] have resulted in the development of atomic absorption spectroscopy as a powerful new tool for the measurement of metals in biological materials. The application of the physical principles of molecular to those of atomic absorption spectro-

scopy greatly increases the sensitivity of the method which can now be exploited for routine analytical purposes[4]. The work recorded here demonstrates that the long path-length absorbing cell permits the direct measurement of magnesium concentrations in serum with a high degree of precision and superb accuracy simply by diluting serum 1 : 50 with water. The repeatability obtained with standard solutions varies from a maximum of 4 per cent at the lowest concentration measured to a minimum of 2 per cent at 1 p.p.m. The repeatability of measuring magnesium in serum varied between 1·7 and 4 per cent, thus the biological content of this fluid in no way affects the determination adversely. The accuracy ascertained by the measurement of certified National Bureau of Standards standards was 100·3 and 102·6 per cent, while recovery of magnesium added to serum was 99 per cent, virtually perfect coincidence. On this basis the serum magnesium concentration of 10 normal persons, determined by this method to be 2·14 ± 0·17 m.equiv. L. can be accepted as representing an absolute analytical mean and distribution.

The concentration of magnesium in seven of the same sera measured by the atomic absorption method was also analysed by flame emission spectrometry. The results obtained by the two methods differed by less than 4 per cent. These results also indicate that the precision and accuracy of the emission method are comparable to the atomic absorption method as judged by measurements of repeatability of National Bureau of Standards standards and of recovery of magnesium added to serum.

The mean of normal serum magnesium concentrations has been the subject of intensive discussion in the literature concerning the 'true' concentration of magnesium in normal human serum[5]. Values as low as 1·5 and as high as 2·2 m.equiv./l. have been stated to reflect precisely the true magnesium concentration of serum; such variations most likely reflect the different analytical methods used[5,7–15]. Unfortunately, accuracy has not been examined in these studies; apparently some authors are under the impression that studies of recovery of an added element can be used as an adequate gauge of analytical accuracy. That this assumption is not acceptable has been documented and discussed thoroughly[15].

The accuracy of the standards used here has been validated directly by comparison with certified samples of the National Bureau of Standards which serve as primary standards of accuracy. Previous conjectures concerning the suitability of certain magnesium salts to serve as standards lack the benefit of experimental validation[4].

Since a number of disease processes can now be related to the diminution[7] or the increase[5] in magnesium in human serum, it should be of considerable general value that the magnesium concentration of normal human serum can be considered to approximate 2·1 m.equiv. l. closely.

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MOLECULAR WEIGHT FRACTIONATION OF POLYANIONS BY CETYLPYRIDINIUM CHLORIDE IN SALT SOLUTIONS

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CETYLPYRIDINIUM chloride and other amphipathic cations are widely used as precipitants for polyanions[1]. The precipitates are soluble in salt solutions at a 'critical electrolyte concentration' (CEC), which depends primarily on: (*a*) the type of cationic group in the detergent[2]; (*b*) the type of anionic group in the polyanion; (*c*) the nature of the salt[3]. Thus, given a particular organic precipitant, polyanion mixtures may be fractionated according to their respective CECs in a given salt solution[4]. In this kind of system it was shown qualitatively by Scott that the molecular weight (M.W.) of the polyanion also influenced the CEC, high molecular weight polyanions having higher CECs than low molecular weight polyanions of similar chemical constitution[4]. Quantitative investigations were first carried out by Laurent *et al.*[5,6], who determined molecular weights of fractions of hyaluronate and heparin obtained according to the CEC principle. A hypothesis of the mechanism of the CEC precipitation was proposed by Scott[7], in which the law of mass action was applied to the equation:

$$P_n + M \quad R \cdot X \quad P_n E^+ \cdot 4 \cdot zM^+ X^-$$

where P_n is a polyanion with n anionic sites, E^+ is a precipitating cation and M^+X^- is the solvent electrolyte. It was concluded that there should be a linear relationship between z and log CEC. Since a true linear polymer built up of a fundamental repeating unit is directly proportional to molecular weight, log CEC should therefore be proportional to 1/M.W. The results of Laurent *et al.*[5,6] fitted this relationship well[8]. This article reports work on keratansulphate, polyacrylate and chondroitin-4-sulphate, to test this relationship further.

Polyacrylate was prepared by neutralizing with sodium hydroxide pooled samples of polyacrylic acid of very varied viscosity, kindly supplied by Imperial Chemical Industries, Ltd., Manchester. The fractions were obtained in sodium chloride solution by the technique of Scott *et al.*[7]. Limiting viscosity numbers (η) were determined on the fractions in 0·2 M sodium chloride solution and are given in Table 1. $(S^2)_D$ was determined by sedimentation equilibrium[9] on two fractions, and these data were used to assume that the modified Staudinger equation can be used for the fractions, the proportionality M.W. $\propto [\eta]^{1.43}$. There seems to be a linear relationship between log CEC and $[\eta]^{1.43}$ as required by the hypothesis (Fig. 1*a*). The scattering of the points around the line in Fig. 1 can be explained by experimental errors.